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HENRY CHANDLER COWLES AND JOHN MERLE COULTER

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- P. 285, line 24, *for* homootypic *read* homeotypic
 P. 285, line 3 from bottom, *for* Fig. 9 *read* Fig. 7
 P. 287, line 7, *for* plates in *read* plates on
 P. 290, magnifications of fig. 1, *for* $\times 330$, $\times 330$, $\times 85$ *read* $\times 500$, $\times 500$, $\times 125$ respectively

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- P. 1, title, *for* ascular *read* vascular
 P. 21, line 34; p. 22, lines 12 and 19, *for* five to numerous *read* five numerical
 P. 31, line 1, *for* quatrata *read* quadrata
 P. 64, line 6 from bottom, *for* 13, *read* 13,
 P. 64, line 4 from bottom, *for* 7. 6 *read* 7. 6.
 P. 85, line 15, *for* and the permanent *read* and to the permanent
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 P. 97, Table III, eleventh heading, insert *j*
 P. 101, line 3, *for* cockelbur *read* cocklebur
 P. 204, line 6 from bottom, *for* humidity of *read* humidity at
 P. 205, line 3, *for* soil surfaces *read* soil surface forces
 P. 207, line 3 from bottom, *for* 1.5 inches *read* 0.15 inches
 P. 239, last line, *for* *Dadoxylon* *read* *Dadoxylon*
 P. 264, last line under HERBACEOUS UNDERGROWTH, *for* mucronata *read* aquilinum
 P. 343, line 5, *for* GROSSWEILER *read* GOSSWEILER



THE BOTANICAL GAZETTE

September 1926

ASCULAR ANATOMY OF RANALIAN FLOWERS^{*}

I. RANUNCULACEAE

GEORGE HUME SMITH

(WITH THIRTY-FOUR FIGURES)

The origin of the angiosperms has long been a question of debate among botanists. One of the chief topics of controversy in this debate has been the nature of the primitive angiospermous flower. To many the external appearance of the Ranalian flower suggests that it is of simple and primitive construction; but, because facts hidden to the student of external morphology and anatomy frequently appear when the internal anatomy is studied, the present investigation was undertaken to determine the exact nature of the vascular anatomy of the Ranalian flower. Members of eight prominent families (Ranunculaceae, Menispermaceae, Calycanthaceae, Anonaceae, Magnoliaceae, Lauraceae, Berberidaceae, and Nymphaeaceae) were selected for study. As the investigation progressed it was discovered that the order consists of several more or less unrelated groups. The groups are: (1) Ranunculaceae, Menispermaceae, and possibly Calycanthaceae; (2) Lauraceae (probably closely related to 1); (3) Berberidaceae; (4) Nymphaeaceae; (5) Anonaceae; (6) Magnoliaceae. Of these five or six groups, the vascular anatomy of the Ranunculaceae is clearly the most simple and least specialized. The present report, therefore, confines itself entirely to a description and discussion of the vascular skeleton of the flowers of typical mem-

^{*} Presented to the Faculty of the Graduate School of Cornell University in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

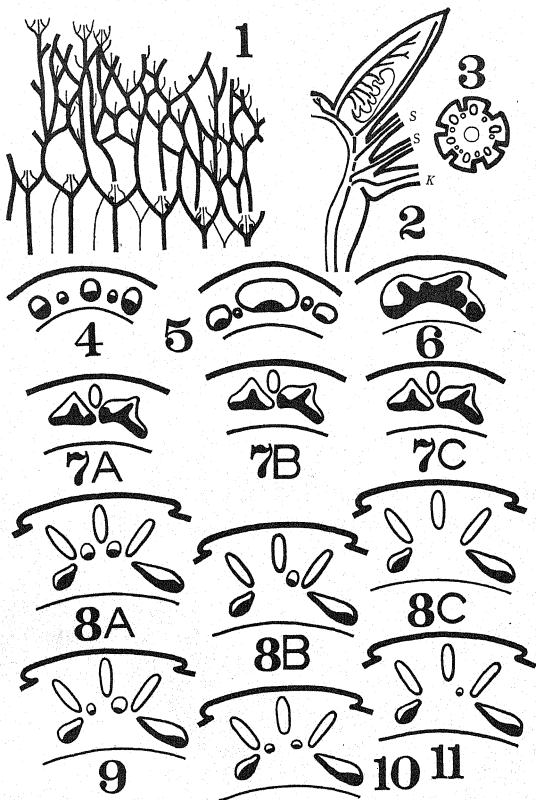
bers of the Ranunculaceae. Subsequent papers will consider the remaining families.

The members of the Ranunculaceae studied are *Caltha palustris* L., *Trollius laxus* Salisb., *Coptis trifolia* (L.) Salisb., *Actaea alba* (L.) Mill., *Actaea rubra* (Ait.) Willd., *Hepatica triloba* Chaix., *Hepatica acutiloba* DC., *Anemone quinquefolia* L., *A. canadensis* L., *Clematis virginiana* L., *Isopyrum bitermum* (Raf.) T. and G., *Anemonella thalictroides* (L.) Spach., *Thalictrum dioicum* L., *Ranunculus hispidus* Michx., *R. abortivus* L., *R. septentrionalis* Poir., and *Aquilegia canadensis* L.

Caltha palustris

ILLUSTRATIONS.—Fig. 1 is a diagram of the course of the vascular bundles upon the unrolled surface of a flower. It is an actual tracing from a single flower. The narrower lines represent the departing traces; the broader lines represent axial strands. The diagram maps completely all vascular tissue in the sepal region. The mapping is incomplete above this level, as the abrupt ending of some of the broader lines (axial strands) indicates. Several difficulties made it impossible to map these regions completely. Three of the carpels are shown in the upper lefthand portion of the figure. Fig. 2 is a median longitudinal section of a flower drawn with the aid of a camera lucida. Fig. 3 is a cross-section of a pedicel. Figs. 4-11 are cross-sections of segments of the receptacle in the region of sepal formation, and are drawn with the aid of a camera lucida. Figs. 4, 5, 6, 7a, 8a are successive levels of one segment. Figs. 8a, 9, 10, 11, and 8c illustrate the derivation of the unilacunar sepal supply from the trilacunar.

DESCRIPTION.—In the peduncle of the flower the vascular tissue is arranged in the form of a hollow cylinder composed of 10-20 separate strands (figs. 1, 3). These strands are of two sizes, large and small. The smaller are intercalated between the larger singly or in pairs. As the slender peduncle gradually swells into the bulbous receptacle (figs. 2, 3-5), the cylinder of vascular tissue increases in diameter proportionately. Coincident with the outward, upward movement of the strands, each large strand gradually becomes broader in the tangential direction, so that the vascular tissue more nearly approaches a continuous ring of tissue. Then each of the

FIGS. 1-11.—*Caltha palustris*: explanation in text

strands, in a definitely spiral succession, gives rise to the entire supply to the sepal facing it (fig. 1). Almost invariably this supply consists of three traces. In the formation of these three traces, the large and tangentially elongated strand facing the sepal divides radially into three (figs. 6, 7). The median one of these three strands departs directly outward and upward through the receptacle into the median portion of the sepal as the median trace to the sepal. Its departure initiates the median sepal trace gap. The two lateral strands remain a portion of the cylinder. Within a distance of less than $10\ \mu$ a projection appears on each of these axial strands (fig. 7*a*, *b*, or *c*). Soon these projections become differentiated into distinct strands (fig. 8*a*, *b*, or *c*). These pass outward and upward as the right lateral and the left lateral traces to the sepal.

As figs. 7 and 8 illustrate, the point of origin of the projections determines the number of separate gaps formed. Thus, in the departure of the three traces to a sepal, one, two, or three separate and distinct gaps in the axial vascular tissue result; and the number of axial vascular strands is two, three, or four. Of these axial strands the two outermost are always large and well developed; the inner ones, when present, may be large and strongly developed, or any size of a grading series until completely absent (figs. 8-11).

In the foregoing statement it has been mentioned that the number of traces to a sepal is three. Occasionally the uppermost and smallest sepal of the series appears to receive but a single strand. Such an illustration would be similar to fig. 8*c*, with the two lateral traces omitted and the base of the sepal somewhat more constricted.

Above the sepal level the axial cylinder shows the following facts. The number of axial strands has been more than doubled by the departure of the sepal traces, and each small pedicel strand has fused or is fusing with its larger axial neighbor. From this circle of strands the first stamen trace arises by the dissection of an axial strand. This dissection occurs at a point almost directly above the position of the first sepal, and at a level slightly higher than that of the last sepal (fig. 1). Similarly the traces to the other stamens appear in a rapid and more or less spiral succession. In the dissection of an axial strand to form a stamen trace, the axial strand splits radially into either two or three parts. If the division is into two parts, one strand remains axial and the other departs outward as

the entire supply to the stamen facing it. If the division is into three parts, the median one of these becomes the supply to the stamen and the two lateral strands remain axial. Whenever this "tri-section" of an axial strand occurs, the number of axial strands is increased. This increase in number of axial strands is counterbalanced by an equally frequent fusion of adjacent axial strands. This constant union of axial strands and subsequent division to form new axial strands and traces is repeated without interruption until the termination of the axis is reached; hence a netlike appearance to the cylinder results.

Following the departure of the trace to the last stamen, the vascular supply to the first carpel arises. The supplies to the other 3-12 or more carpels arise in a rapid spiral succession. The supply to each carpel consists of three separate traces. Each trace originates directly from the axial cylinder. In the supply to the individual carpel the dorsal trace arises first. An axial bundle segments radially into three strands. The median of these departs from the axis and passes up the dorsal margin of the carpel. At a point on the axial cylinder very slightly above the point of departure of the dorsal trace, each of the two axial strands which have resulted from the departure of the dorsal carpel trace divides radially into two strands. Of these the one nearer the dorsal trace gap becomes a ventral trace. Some axial strands definitely and abruptly terminate with the departure of the last carpel trace arising from that particular strand, but the majority do not. The axial strand continues upward, but quickly frays and rapidly fades away within a distance of 10-20 μ . In fraying, the few spiral elements wander toward the tip of the cone-like receptacle (figs. 1, 2).

Within the carpel the three traces act in a very characteristic manner (figs. 2, 28). The dorsal trace passes unbranched up the dorsal margin of the carpel and fades as the stigma is approached. The two ventral traces pass up along the ventral suture and fade away within the stylar region. In the lower portion of its course, each ventral trace gives off inwardly a series of strands to the number of about ten. Each of these strands enters one ovule of the row of ovules situated directly under this trace.

DISCUSSION.—Three important features of the axial skeleton as a whole are to be noted. (1) The skeleton appears as a hollow cylindrical network of strands bristling with departing traces. Thus it

discloses a similarity to the vascular design of a vegetative axis. (2) The spiral insertion of the floral organs upon the axis emphasizes the similarity between vegetative and floral axes. (3) The fact that each trace to each organ emerges from the cylinder and passes directly outward and unbranched to the organ, designates the floral axis as entirely primary. If the floral axis (receptacle) were an inflorescence, each floral trace would display some evidence of nodal differentiation.

The apical portion of the floral axis also deserves special consideration. The presence of remnants of vascular tissue above the level at which the last carpel trace has departed indicates that the most apical of the floral organs have entirely disappeared. The reasons for this conclusion are three. (1) Vascular tissue, historically, always terminates in some spot where it is physiologically valuable. (2) In the consideration of evolutionary series, it has been found that vascular tissue never arises in advance of the structure it is to supply. (3) The vascular tissue to an organ, on the other hand, may remain after the organ itself has disappeared. Thus *Calltha palustris* can be added to the list of those forms showing a suppression of floral organs.

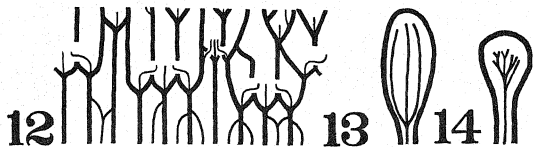
In the description of the sepals, it has been pointed out that many sepals receive three traces arising so that three separate gaps are formed, and that a grading series can be constructed from this type to one culminating in the departure of three traces from a common gap (figs. 8a, 9, 10, 11, 8c in the order named). The justification in reading the series in this direction lies in the fact that a general tendency exists among the angiosperms toward a reduction in the amount of woody tissue. Herbaceous forms, for instance, have been derived from woody forms.

The carpels are three trace multiovulate structures. As the discussion progresses and other carpellary conditions are described and explained, it will become apparent that this three trace multiovulate condition is primitive.

Trollius laxus

DESCRIPTION.—This flower is constructed upon the same general plan as *Calltha*. It differs in five respects. (1) Ten to twelve large strands and at least an equal number of smaller alternating strands

are present in the peduncle (fig. 12). (2) Each of the 4-15 sepals receives three traces. These traces are derived from the simultaneous division of three large axial strands after the manner shown in fig. 12. Note that three distinct gaps are always formed. (3) Above the sepals 15-25 small club-shaped "petals" are present. These are arranged spirally, receive one trace each, and this spiral merges into the spiral of the stamens. (4) Within the carpel, strands pass from the ventral traces to the ovules, one to the ovule; and, in addition, above the ovules strands pass inward from the ventral traces to the ovulatory cavity and end abruptly (fig. 27). (5) No fraying of the termini of the axial strands of the receptacle or other evidence of the suppression of terminal carpels exists.



FIGS. 12-14.—Fig. 12, *Trollius laxus*: vascular system of axis of sepal and lower petal region of flower, split open and spread out in one plane; fig. 13, petal; fig. 14, *Coptis trifolia*, petal.

DISCUSSION.—*Trollius*, therefore, presents two new problems. One considers the evidence of the staminodal affinity of the petal; the other deals with the possibility of the presence of suppressed ovules.

The sepals and petals are clearly unlike in external appearance. The sepal is broad and expanded, and with an unstricted base. The petal is small and inconspicuous, and the basal portion is slender and filamentous. In vascular anatomy these two organs also differ radically. The number of traces entering a sepal is three. Within the sepal these branch profusely and the sepal becomes thoroughly net-veined. A single trace enters the petal. It does not branch until it enters the expanded portion, and then it subdivides into three prongs, which extend parallel and unbranched to the apex of the organ (fig. 13).

On the other hand, the petal resembles the stamen in several respects. The club-shaped appearance with the long and slender filamentous basal portion makes the resemblance to stamens noticeable. Again, the vascular anatomy is indistinguishable from that of the stamen, not only because both are organs receiving a single trace, but also because the first stamen trace departs from the axis immediately after the last petal trace and in direct continuation of the spiral order. Thus external similarity and internal anatomy identify the petal with the stamen.

The behavior of the vascular tissue within the carpel gives ample evidence that the carpel of *Trollius* has been derived from a form having a larger number of ovules. As fig. 27 illustrates, a series of strands is given off from each ventral trace toward the ovular cavity. The lower of these enter ovules, but the upper ones end abruptly at the margin of the cavity. Thus the form, course, and position of these traces suggest that they are supplies to suppressed ovules. Again, if these strands are not traces to ovules, what can be the original physiological significance of their presence? What tissue could they have been intended to supply? They cannot be the forerunner of an increase in the number of ovules of the carpel, because vascular tissue has never been known to appear as a forerunner to the evolution of an organ. On the other hand, vascular strands may remain lagging behind after the organ has disappeared; therefore the anatomical evidence points to the conclusion that these traces are supplies to suppressed ovules.

The theory that these traces are supplies to suppressed ovules gains additional support from other morphological data concerning Ranunculaceae. ERNST BESSEY (7) discovered one or more rudimentary ovules present above the functional ovule in *Anemone caroliniana* Walt., *A. canadensis* L., and *Pulsatilla hirsutissima* (Pursh.) Britt. In some cases these ovules were represented only by a few-celled rudimentary archesporium, the presence of which would not have been discernible to one not studying the comparative development of pistils. Again, GUIGNARD (10), in his investigations of the embryo sacs of angiosperms, figures a specimen of *Clematis cirrhosa* showing one fully developed ovule, and above it two partially de-

veloped ovules. BAILLON (2), in his discussion of the Ranunculaceae, found accessory ovules so common in *Anemone* that he concluded that the number was actually five, but four had degenerated and the fifth, at the expense of the others, remained. From the standpoint of systematic evidence, C. E. BESSEY (6) argued and proved the dictum "uniovulate from multiovulate." Thus the vascular anatomy of the pistil of *Trollius laxus* confirms and strengthens the contention that the evolutionary tendency has been toward a gradual reduction in the number of ovules contained in a pistil.

Coptis trifolia

DESCRIPTION.—*Coptis* differs anatomically from *Trollius* in but seven minor respects. (1) The peduncle contains only about seven vascular strands; usually five are large, and the remaining smaller and either lignified or unlignified. (2) In sepal trace formation the broadened axial strands split radially into two to six strands. (3) The divisions of these strands are such that each sepal receives three traces, and the number of axial bundles is increased to above ten. (4) The divisions are so rapid that no definitely spiral order can be discerned. On the other hand, the arrangement is not clearly cyclic. (5) Above the sepal level the axial bundles are not clearly set apart from each other, because the stamen traces depart in very rapid succession and the gaps are quickly closed. (6) Within the carpel the ventral traces give rise to no strands which do not supply ovules. Thus the ventral traces do not show evidence of supplies to suppressed ovules. (7) The trace entering the "petal" gives rise to several small lateral branches in the more expanded bladelike portion (fig. 14). Fundamentally, however, the "petals" are merely modified stamens as in *Trollius*.

Actaea

DESCRIPTION.—In *Actaea alba* the vascular anatomy of the flower up through the stamen level is very similar to that of the preceding genera. A cylinder of strands variable both in number and in size enters the receptacle. At the first division of these strands the sepals are supplied with three traces each. These traces arise as

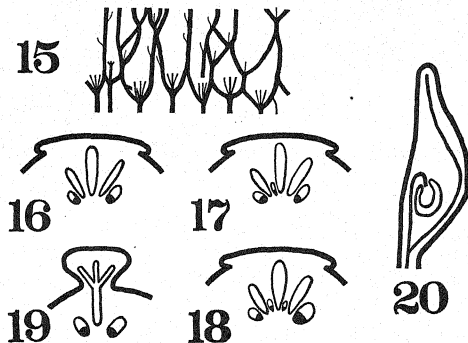
three radiating fingers from a common gap. The traces to the petals and the stamens are one each. Consequently the petals are indistinguishable from the stamens, so far as the vascular supply is concerned. Above the stamen level certain interesting differences occur. After the last stamen trace has departed, the five or six axial strands rapidly coalesce into three. These three strands continue up the axis in this formation to the base of the single carpel. Within the carpel the median of the three strands becomes the dorsal trace; the two lateral ones become the ventral traces. The dorsal trace passes up the dorsal margin of the carpel, and early in its course gives rise to a few fine lateral branches, but itself remains strong to the stigmatic region. The two ventral traces give rise to occasional branches which ramify the marginal tissue toward the dorsal portion of the carpel. At the same time they give off inwardly individual branches to the two rows of ovules.

DISCUSSION.—The vascular plan of the receptacle above the stamen level raises three questions. (1) Does the interval between the last stamen and the pistil represent a single elongated internode, or does it represent the pistillate portion of the receptacle with all pistils suppressed but one? The vascular cylinder of this region gives off no blind traces, therefore the presence of intervening pistils cannot be assumed. On the other hand, not only the intervening pistils but also the evidence of vascular supplies to them may have disappeared. (2) Is the pistil terminal? If it were pseudo-terminal, the ends of the axial vascular strands of the receptacle would probably show frayed projections beyond the departure of the pistil supply such as *Caltha* displays. (3) Is the pistil a single carpel? The number of strands entering the base of the pistil is three. Of these two are ovule-bearing (ventral) and one is dorsal. This fact (three strands) makes it a simple pistil, because throughout all of this investigation of the Ranales and all records mentioning the number of traces entering the angiospermous carpel the number is three, or a modification of this number. A pistil, if compound, will show anatomical evidence of receiving more than one dorsal and two ventral traces. The truth of this statement will be expanded in the consideration of the Berberidaceae.

In *Actaea rubra* the floral anatomy is precisely that of *A. alba*.

Hepatica

DESCRIPTION.—In *Hepatica triloba* the course of the 10-12 strands through the peduncle and the receptacle (fig. 15) is very similar to that of *Caltha*, except that the bundles are not all of uniform size. At least 6-9 are large and strongly lignified; the remainder are smaller. Some or all of these smaller strands are unlignified. These smaller strands join their larger axial neighbors at about the level of sepal trace formation, or completely fade away at about this level.



FIGS. 15-20.—*Hepatica triloba*: fig. 15, diagram of vascular system of axis of sepal and lower stamen region of flower spread out in one plane; figs. 16-19, cross-sections to show modes of lateral sepal trace departure; fig. 20, carpel.

In sepal trace formation the large axial strand facing a sepal elongates tangentially and splits radially into 3-7 strands. Commonly the segmentation is into five strands. The median three of these strands depart simultaneously from the cylinder as three spreading fingers, and enter the sepal as the three traces to the sepal (fig. 16); thus but one gap is formed. Occasionally an axial strand divides into six strands instead of five. As a result, one lateral sepal trace and the median sepal trace enjoy a common gap, whereas the other lateral trace enjoys a distinctly individual gap (fig. 17). This

distinctly individual gap may or may not be quickly closed by the union of the two adjacent axial strands. Even less frequent is the segmentation of the axial strand into seven. The two lateral sepal trace gaps are thus separated from the median sepal trace gap by axial vascular tissue (fig. 18). Another variation frequently occurs whenever the number of sepals is over five or six. The smaller and higher sepals receive but a single trace. In the derivation of the supply to such sepals, the tangentially expanded axial bundle facing the sepal divides into three parts; the two lateral remain axial, the median passes into the sepal as an undivided unit. Immediately upon entering the sepal, a subdivision of this unit into three strands occurs (fig. 19).

The first traces arising above the sepal level pass to the stamens, one to each stamen. After the stamens are supplied, the traces which arise from the axial framework pass to the carpels, one to each carpel. The axial strands terminate as traces to carpels. That a single strand enters a carpel, instead of three, is an important distinction between this genus and the foregoing genera.

Within the carpel the course of the vascular strand (figs. 20, 33) differs from that of *Caltha*. In the base of the carpel the strand splits into two portions. One follows the dorsal margin to the stigma, the other proceeds directly up into the single ovule.

The vascular anatomy of the flower of *Hepatica acutiloba* is identical with that of *H. triloba*.

DISCUSSION.—As in *Caltha*, the behavior of the vascular strands at the level of sepal trace formation again gives evidence that the three trace, single gap condition is derived from a three trace, three gap condition (compare figs. 16-18 with figs. 8a, b, and c).

Consider now the higher smaller sepal which frequently receives but a single strand. Because the majority of *Hepatica* sepals receive three traces arising from a common point (fig. 16), the definitely three prong division of the single strand, such as enters the type of sepal under consideration (fig. 19), suggests an unusually close adherence of the lateral traces to the median. Thus in *Hepatica* a second tendency of sepal traces is manifest, namely, lateral fusion of adjacent traces to form a single strand.

The carpel receives but a single vascular strand and contains but

a single ovule. The full significance of the fact that a carpel receives but one trace and contains but one ovule cannot be appreciated until the carpels of *Ranunculus*, *Aquilegia*, and *Calycanthus* have been considered. A discussion, therefore, of the origin of the single trace, uniovulate carpel is deferred.

Anemone

In all essential respects *A. quinquefolia* and *A. canadensis*, both studied by the writer, and *A. coronaria* studied by HENSLow (12) are of the same fundamental design as *Hepatica*. Likewise, *Iso-pyrum bilernatum* is very similarly constructed.

Clematis

In *C. virginiana* each sepal receives three or four traces. Each of these traces arises from a separate point on the vascular cylinder and leaves a separate gap. This is the most striking and only difference of importance from *Hepatica triloba*. HENSLow's (12) description of *Clematis vitalba* L. shows that it differs from *H. triloba* in a similar respect. As *Clematis* is the only genus studied of this family in which the number of sepal traces is more than three, no direct corroborative anatomical evidence is at hand upon which to build any explanations for this condition.

Anemonella thalictroides and Thalictrum dioicum

DESCRIPTION OF ANEMONELLA.—In all respects not specifically mentioned, the design of the vascular framework of a flower of *Anemonella thalictroides* (fig. 21) is similar to *Hepatica*. The pedicel most frequently contains seven equal sized, equidistant vascular strands. In some cases this number increases to eight or nine in the base of the receptacle by the radial division of one or two of the strands. The subdivision of the axial vascular strands during the process of sepal trace formation displays a greater variation than in the genera previously described. A strand splits radially into 2-5 strands, 1-3 of these departing from the cylinder as sepal traces. The other remains axial. Most sepals receive three traces, although two trace sepals are not infrequent. The three traces to a sepal may arise from one, two, or three separate and distinct axial strands. The number of distinct

gaps formed by the passage of traces to a sepal is 1-3, according to the mode of origin of the individual traces (fig. 21). The strands which remain axial give rise to the stamen and the carpel traces.



21



22



23

FIGS. 21-23.—Fig. 21, *Anemonella thalictroides*: diagram of vascular system of axis of sepal region of flower spread out in one plane; figs. 22, 23, *Thalictrum dioicum*: similar diagrams of staminate and pistillate flowers respectively.

Within the basal portion of the carpel the trace branches into a dorsal and a ventral strand, as in *Hepatica*, but each of these in turn subdivides until every rib of the carpel is supplied with a vein. The carpel of *Anemonella* is 8-10-ribbed. Of the veins of ventral origin, the largest and most prominent forks into three strands after it has traversed about two-thirds the length of the carpel wall. Of these three strands the two lateral ones continue up the carpel wall to the style and fade away; the median one of the three curves inward and downward to the ovule.

DESCRIPTION OF *THALICTRUM DIOICUM*.—In the staminate flower of *T. dioicum* (fig. 22) the pedicel contains about eight vascular strands. As they approach the level of sepal trace formation, each strand expands tangentially and splits radially into 2-5 strands. Some of these are sepal traces and each of the 4-5 sepals receives 1-3 traces. Eight to sixteen strands remain axial after the sepals are supplied. These quickly divide into a number of strands equal to the number of stamens, and a strand enters

a stamen as the total supply to that stamen. The vascular anatomy gives no hint of a terminal carpellary region.

The plan of the vascular skeleton of the carpellate flower of *T. dioicum* (fig. 23) is practically the same as that of the staminate flower. The axial strands in the pedicel and lower receptacle are usually four. These split into a variable number of strands. Of the

resulting strands, some pass to the sepals, 1-3 to the individual sepal, and the others remain axial. After sepal trace formation, the axial strands, with the possibility of an occasional union of two adjacent strands, quickly separate into a number of traces equal to the number of carpels (about 9). Within the carpel (fig. 34) the trace divides into two strands; one bears toward the dorsal side of the carpel and the other to the ventral. These extend upward along diametrically opposite ribs of the carpel. As soon as the dorsal and ventral strands have become differentiated, each of them gives off lateral branches. Some of these in turn subdivide until every rib of the carpel has received a vein. At a still higher level the ventral strand proper gives rise to another branch. This curves inward and downward to the ovule. Near the base of the style the two rib branches nearest the ventral strand proper reunite with it, and this vein extends upward to the stigmatic region. The remaining rib branches derived from the ventral strand and the majority of those of dorsal origin fade away at the base of the style. The dorsal strand proper and a certain few of its derivatives reach the stigmatic region.

DISCUSSION.—In the sepal receiving three traces, the one is median and the others lie, one on the right side and one on the left side of the median trace; whereas in the sepal receiving two traces, one trace occupies the median position, the other that of one of the lateral traces, and a distinct vacancy occurs on the other side where another lateral trace could occur. Furthermore, within the sepal the first branch of the median trace assumes the burden of this missing lateral trace. Thus it is clearly evident from the anatomical facts as stated that the basic plan is three traces to the sepal in *Anemonella* and *Thalictrum*.

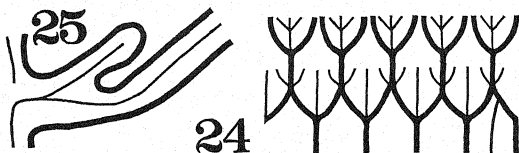
The carpels of *Thalictrum* (and *Anemonella*) receive but a single strand and contain but one ovule. In this respect these forms are similar to *Hepatica*. The branching, however, in each is more extensive than in *Hepatica*, and the ovule is not so nearly basal.

Thalictrum dioicum is the only species of the Ranunculaceae studied in which the flowers are both structurally and functionally imperfect. The anatomy discloses no conclusive evidence that the flowers were once perfect. In the staminate flowers, for instance, the terminal portion of the axial cylinder gives no indication of the abor-

tion of a terminal carpellary region such as the carpellary region of *Calltha palustris* might lead one to anticipate. The pistillate flower also gives no indication of a suppression of a staminate interval.

Ranunculus

DESCRIPTION.—In *R. hispidus* the number of vascular strands of the peduncle is either six or seven. Five of these are large, distinctly lignified, and equidistant from one another. The five sepals are directly above them. One or two smaller, yet distinctly lignified strands are also included in this circle in such a manner as always to be separated from each other by at least one large strand. As the sepal level is approached, each of the five large bundles splits radi-



FIGS. 24, 25.—*Ranunculus*: diagram of strands of vascular system of axis of sepal and petal region of flower; fig. 25, petal and attached nectariferous scale (*R. septentrionalis*).

ally into three (fig. 24). The median one of the three thus formed advances outward and upward through the receptacle, and becomes the median trace to the sepal facing it. By the departure of these five traces, the nearly complete cylinder of tissue is broken up into five groups of bundles. The members of a group converge and unite to form five bundles (fig. 24). Either just as this union into five bundles is occurring or just after it has been consummated, traces are given off from the vascular cylinder in such a manner that each sepal is supplied with a right and a left lateral trace (fig. 24). Note that the three traces to a sepal enjoy a common gap. At a slightly higher level the petals are supplied with one trace each (fig. 24).

As the petal trace passes outward through the parenchyma, which is transitional between the receptacle and the petal, it divides into three strands. These are the three primary veins of the petal, and

initiate its palmate venation. No vascular supply passes up into the small scale situated at the base of the petal. With the departure of the petal traces the number of axial strands is ten. These are equally distant and of uniform size. Immediately stamen traces begin to emerge from these axial strands after a fashion similar to that described for *Calltha*. It is also to be noted that the division of the axial strands is not simultaneous (cyclic) but spiral.

As in the preceding genera, the vascular cylinder of the floral axis becomes reticulate in appearance, due to the constant division and recombination of axial strands. With the departure of the last of the stamen traces, the axial strands arrange themselves into a more compact and less netlike cylinder, the gaps becoming fewer and less distinct. The sheathing parenchyma becomes very nearly a continuous enveloping ring. This condition continues through the short axial region which can externally be seen to separate the stamens and carpels. At the termination of this interval, the first carpel trace appears as a projection from the almost complete cylinder of vascular tissue. By the departure of the traces to the four or five following carpels, the vascular cylinder is dissected into numerous small axial strands, thus assuming again a distinctly reticulate appearance. From this type of axial cylinder the remaining carpel traces arise after a manner precisely analogous to that of the stamen region. One trace enters each carpel.

The course of the strand within the carpel is very definite (fig. 32). The single entering strand soon divides into two, one of which bears quickly to the dorsal side of the carpel, and extends upward and unbranched to the base of the stigma. This is the dorsal strand. The other (ventral strand) immediately forks into three portions, which advance directly upward, paralleling one another. At about one-fourth the length of the carpel wall, the median of these three strands turns abruptly inward and enters the ovule. A short distance above the ovular cavity each of the lateral strands sends a side branch through the carpel wall in the direction of the dorsal margin. Either or both of these side branches curve extensively enough to meet the dorsal strand. Sometimes the two lateral portions of the ventral strand end blindly at the base of the style; at

other times they come in contact with each other and then fuse with the dorsal strand; in still other carpels just one of the ventral strands fuses with the dorsal and the other ventral strand ends blindly.

Ranunculus abortivus differs from *R. hispidus* in just one respect, the two ventral strands of the carpel are merely two tiny stubs. As in *R. hispidus*, no vascular supply passes up into the small scale at the base of the petal.

The difference between *Ranunculus septentrionalis* and the other two species is due probably to the fact that the flower is considerably larger. Usually two, three, or even four small lignified bundles are present in the peduncle in addition to the five large strands. Again the lateral sepal traces are not given off as soon after the median sepal trace as in the other two species. It is also of interest to note that the first branch of the median vein of the petal arises from the ventral surface of the vein and passes up into the small scale situated at the base of the petal (fig. 25).

The vascular supply within the carpel is also slightly different from that of *R. hispidus*. The two ventral strands never branch extensively; usually just one of them gives rise to a branch which passes laterally.

In all three species studied occasional variations occur within the sepal region. An occasional sepal receives three traces, either arising so that three visible gaps are formed or only two. In this latter case the gap formed by the lateral trace alone is quickly closed by the reunion of the strands. In another type of variation, one or rarely both lateral traces are lacking.

DISCUSSION.—From the data just presented, the sepals of *Ranunculus*, as those of *Caltha* and *Hepatica*, give evidence of an ancestry in which the three traces originally arose from three separate and distinct gaps.

The affinities of the petals are not easily seen. The petals of *Ranunculus* could be considered as sepals, with the three traces completely fused to the base of the organ; or the petals could be considered as sepals in which the two lateral traces have disappeared. While such possibilities of a relationship to sepals do exist, several facts point to the petal as an organ distinctly different from the sepal. The strongest evidence of a lack of relationship lies in the pres-

ence of the nectariferous scale at the base of every petal and its complete absence from every sepal. The fact that the nectariferous scales of two of the species studied receive no vascular supply suggests that the scale is an evagination of the petal itself. The fact that the vascular supply to the nectariferous scale of *R. septentrionalis* arises from a vein of the petal definitely confirms the conclusion that the nectariferous scale is not an independent organ secondarily fused with the petal, but actually is a part of the petal.

Certain facts suggest the stamen affinity for the petal. The presence of but a single strand is one of these; but, as was pointed out, two other possibilities exist as to the nature of this single strand. Additional facts, however, strengthen the possibilities of the stamen affinity of the petal. Whereas the number of sepals is always five and whorled, the petals are occasionally more than five and spirally arranged. Furthermore, when the arrangement is spiral, it is continued by the stamens without the slightest break. Thus the arrangement of the petals in these instances suggests a stamen affinity.

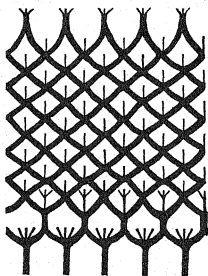


FIG. 26.—*Aquilegia canadensis*: diagram of strands upon unrolled surface of vascular system of axis of flower, split open and spread out in one plane.

Aquilegia canadensis

DESCRIPTION.—The number of large vascular strands in the peduncle is five. Sometimes one or two smaller strands are present. In the lower receptacle the five large strands simultaneously segment radially into five parts each. The median three of each group of five pass outward and upward through the receptacle as three diverging fingers, and enter the sepal facing as the three traces to that sepal. The adjacent derivatives of two contiguous axial large strands unite on radii alternate with those occupied by the large pedicel strands at the time of sepal trace formation (fig. 26). These five strands soon divide radially into three each. As in *Ranunculus his-*

pidus, the median strand of each group of three departs as the total supply to the petal facing it. As this trace passes through the transitional zone from receptacle to petal, it branches into three parts. These three parts are the three primary veins of the petal. After a fashion analogous to that whereby the petal traces were formed, traces to the alternating whorls of five stamens each are formed. The single trace entering the individual stamen never branches.

The ten axial strands resulting from the departure of the last whorl of stamen traces pass up the axis in pairs as distinct strands for an appreciable distance. Then the two members of a pair unite only to redivide at once into three strands each. The three strands compose the supply to the carpel facing. Thus each carpel receives three separate traces, which are distributed so that the two lateral strands are the ventral traces and the median one is the dorsal trace to the carpel. The branching of these strands within the carpel is practically the same as in any of the flowers of the Ranunculaceae in which the carpels are supplied with three traces (fig. 28). These genera are *Callitha*, *Trollius*, *Coptis*, and *Actaea*.

In one specimen examined an interesting variation occurs in the derivation of the carpel supply (fig. 31). The ten axial strands resulting from the formation of the traces to the last whorl of stamens do not remain as ten distinct strands, but coalesce to form a definite hollow cylinder before reaching the carpel level. At this point the cylinder breaks up into three distinct bundles, and each bundle enters one of the three carpels. This specimen has but three carpels. In the base of each carpel the trace divides into the usual dorsal and two ventral strands. Thus these particular three carpels display characteristics common to such forms as *Callitha* on the one hand, and *Ranunculus* on the other. The single entering trace and its subdivision into dorsal and ventral strands is similar to the conditions in the achene of *R. hispidus*, which receives but a single strand; the branching of each ventral trace to supply a row of ovules is like that of *C. palustris*.

DISCUSSION.—*Aquilegia* is the only genus studied in which the numerical plan is five throughout and all parts are inserted in whorls. To prove conclusively the derivation of the whorled condition from the spiral, anatomical evidence should reveal three distinct steps.

The first step is one in which the floral axis is greatly compressed. Accompanying this vertical compression of the axis the "phyllotaxy" becomes high. As the evidence of the other *Ranunculaceae* studied reveals, the "phyllotaxy" of the stamen-carpel region is always too high to determine satisfactorily, and thus suggests a marked vertical compression. The second step in the derivation of the whorled condition from the spiral involves a still more pronounced vertical compression of the axis and a dropping out of some of the organs. Thus the compression of the axis and the disappearance of segments of each cycle of the spiral would cause the remaining organs to appear as inserted essentially in whorls. It is upon the question of the disappearance of some of the floral organs, the most essential feature of this second step, that the anatomical evidence is entirely lacking. No blind traces leading off from the axial cylinder and suggesting a connection with suppressed sepals, stamens, or carpels, interspersed among the surviving ones have been found. It is to be recalled that ample evidence was found for the suppression of ovules (*Trollius laxus*), and of the terminal carpellary portion of the axis (*Caltha palustris*), and other investigators (3, 4, 8, 16) have found remnants of vascular tissue to suppressed perianths, stamens, and other floral structures. The third step in the possible derivation of the whorled condition from the spiral involves a still greater shortening of the internodes, so that the remaining floral organs come into exact whorls. In other words, certain internodes become so short as to come into coincidence. *Aquilegia* itself may be taken as an illustration of this condition. Thus, so far as the anatomical evidence itself is concerned, only the spiral and strictly whorled conditions exist in the *Ranunculaceae*. The high phyllotaxy and evident vertical compression of the axis, however, are sufficient to suggest that the intermediate condition just discussed has historically existed in the derivation of the strictly whorled condition of *Aquilegia* from the spiral condition of the more primitive *Ranunculaceae*.

The anatomical proof is also somewhat intangible concerning the possible derivation of the five to numerous arrangement of floral organs from the indefinite arrangement so common in the other genera. Certain interesting anatomical facts, however, exist in this connection. Although in no species studied does the number of

axial strands of the pedicel consistently equal the number of sepals, in *Coptis* and *Anemone* the number of large pedicel strands is usually five, in spite of the fact that the number of sepals may be greater or less than this; and, in *Ranunculus* and *Aquilegia*, the number of large pedicel bundles is always five. Therefore in view of the fact that the smaller intercalated bundles found in *Anemone* suggest and actually show evidence that they are vanishing, and in view of the fact that in the majority of families in which a definite numerical plan exists the number of bundles is in harmony with the number and arrangement of the floral parts, the inference is that a tendency exists among certain genera of Ranunculaceae toward a completely five to numerous plan.

Another point of interest is the consideration of the sepal. The three traces to a sepal depart from the common gap. In this respect they are identical with the supplies to the majority of the sepals of *Hepatica triloba* (fig. 16). The condition differs from that of *Hepatica*, however, in that the individual sepal always receives three traces, always arising from a common gap, and in that the number of sepals is always five and whorled. In these last two respects (five to numerous and whorled) the sepal condition is similar to that of *Ranunculus*. Thus the sepals display a combination of certain characteristics found in the *Hepatica* and the *Ranunculus* type of flower.

The supply to the petal is identical in appearance with that of the normal five petal flower of *Ranunculus*. The petals are probably stamen-like, therefore, for the same reason that the petals of *Ranunculus* are stamen-like. The innermost stamens are scalelike and are sometimes described as scales. They are nevertheless distinctly stamens; both the similarity of vascular anatomy and the presence of underdeveloped pollen sacs are proof of this.

The carpels are multiovulate and usually receive three traces. The conditions, therefore, are similar to those of *Callitha palustris*. In one specimen described, however, the vascular tissue entering a carpel is in the form of a single strand. The evidence is in favor of considering this a modification of the distinctly three trace condition in which the three strands are fused laterally up to the base of the carpel.

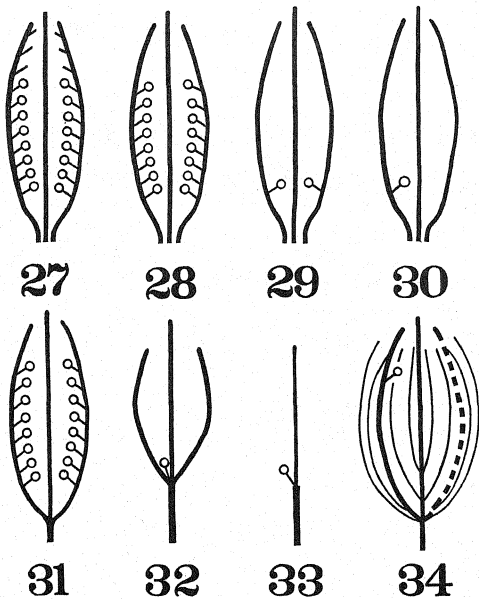
Conclusions

FLORAL AXIS.—The receptacle and pedicel, as a unit of structure, were considered in the discussion of *Caltha*. The description of each succeeding form has revealed similar characteristics. (1) The floral vascular skeleton is a pear-shaped network of strands studded with the departing traces. (2) Every trace passes directly and unbranched to an organ, thereby indicating that the axis is a simple flower and not a telescoped inflorescence. (3) The floral organs are generally inserted spirally. (4) The axis is divided vertically into three regions: carpel, stamen, sepal.

CARPEL.—In a general review let us first consider the vascular anatomy of the carpel of *Trollius laxus* (fig. 27). Three traces enter the carpel, a dorsal and two ventral. Each ventral trace supplies a row of ovules with one strand each, and gives off three or four additional strands above those to the ovules. These strands, according to the discussion of *Trollius*, are the supplies to completely suppressed ovules. If it is to be assumed that these strands themselves ultimately disappear, the resulting condition is portrayed by fig. 28. This figure also shows the vascular plan of the carpels of *Caltha*, *Coptis*, *Actaea*, and *Aquilegia*. Continuing the progressive suppression of ovules and subsequent disappearance of vascular strands, a condition is reached in which each ventral trace supplies but one ovule (fig. 29); and finally a condition is reached in which but one ventral trace supplies a single ovule (fig. 30). The two conditions just mentioned are actually found in *Calycanthus floridus* L. This species, as a future paper will discuss, is closely related to the Ranunculaceae. Fig. 31 illustrates another type of variation from figs. 27, 28. It represents the unusual carpel of *Aquilegia*. From an inspection and comparison of this figure with fig. 28, it is evident that the single strand represents the two ventral traces fused to the dorsal. Such a lateral fusion of traces cannot be considered extraordinary for two reasons. In the discussion of *Caltha*, *Hepatica*, and *Ranunculus* mention has been made of the lateral fusion of the three sepal traces to form a single strand.

Again students (15) of nodal anatomy of vegetative axes have pointed out that the three traces to a leaf may adhere from the point

of origin into the base of the leaf, thereby forming a single strand. Fig. 32 represents a carpel of *Ranunculus hispidus*. In this carpel the three traces are united from their point of origin into the base of



FIGS. 27-34.—Series of diagrams illustrating derivation of uniovulate single trace carpel from multiovulate three trace carpel: fig. 27, *Trollius*; fig. 28, *Caltha-Aquilegia*; figs. 29, 30, *Calycanthus*; fig. 31, *Aquilegia* (unusual); fig. 32, *Ranunculus*; fig. 33, *Hepatica*; fig. 34, *Thalictrum*.

the carpel, and the number of ovules has been reduced to one. Thus in this carpel the two conditions expressed separately in figs. 30 and 31 are found; namely, reduction of ovules from many to few to one,

and union of three traces to form a single strand. Fig. 33 (see also fig. 20) represents a condition in which the two ventral traces never become separated from the dorsal trace. Such a diagram depicts the conditions in *Hepatica*, *Anemone*, and *Clematis*. Two other genera, *Thalictrum* and *Anemonella*, show a slightly different type of variation from the *Caltha-Trollius-Aquilegia* type. The three traces are united into the basal portion of the carpel (fig. 34). The dorsal strand becomes a distinct strand and passes up the dorsal margin of the carpel. The two ventral strands remain as a single unit and pass up the ventral margin. Both dorsal and fused ventral strands give off lateral branches in greater numbers than any of the other Ranunculaceae. The strand passing up the ventral margin of the carpel represents the fusion of two ventral strands, because in no other form studied does a ventral trace give off branches laterally both to the right and left. Fig. 34 shows this strand as resolved into its two components. It is also to be noted that the trace to the ovule arises at a point some distance above the point of separation of the dorsal and ventral strands. This is indicative of the fact that the surviving ovule is not a basal one.

Thus anatomical studies of the carpels of the Ranunculaceae (figs. 27-34) reveal two important conclusions. (1) The two ventral traces to a carpel may become fused to the dorsal trace, thereby forming a single entering strand. (2) The uniovulate carpel can be derived from the multiovulate carpel through a gradual suppression of ovules. This conclusion is especially interesting because it is derived independently of the morphological and taxonomic evidence presented in the discussion of *Trollius* to point to a similar conclusion.

STAMEN.—The stamens, not only of the Ranunculaceae but also of angiosperms in general, so far as recorded in literature, are distinctly single trace organs. In the Ranunculaceae absolutely no branching of this trace occurs; therefore neither a pinnate nor a palmately veined ancestor can be assumed. Furthermore, this single trace gives no evidence as to whether it is a morphological unit, the remnant of a more extensive supply, or a fusion of several traces. An interpretation of the vascular condition of the stamen, therefore, is not possible on the evidence available. Thus the vascular anatomy of the staminate structures of some of the other groups of the

"primitive" dicotyledons and monocotyledons offers a field for interesting investigations in the hope of solving the problem.

PETAL.—When the anatomical data concerning the petals of the Ranunculaceae are considered, the corolla disappears as a morphological entity. The petals are sterile stamens. The evidence does not reveal the stages of this change. The suggestion is made that the petal could be derived from an expanded leaflike microsporophyll which has retained the leaflike form but lost the sporophyllaceous character; and the stamen as retaining the sporophyllaceous character but losing the leaflike form. Another possible derivation of the petal is through a loss of the anther of the typical stamen and a subsequent dilatation of the filament. More data concerning the exact nature of the stamen itself, however, must be gathered before the mode of origin of the petal of the Ranunculaceae from its microphyllous ancestor can be determined. It is also to be emphasized that, whereas the petals of the Ranunculaceae are of stamen affinity, no indication exists but that the petals of some other plant groups may be sepaloïd in nature rather than petaloïd.

SEPAL.—In the discussion of *Caltha palustris* it was pointed out that a series could be constructed from sepals receiving three traces arising from three distinct gaps to sepals receiving three traces from a single gap (figs. 8a, 9, 10, 11, 8c, in the order named). The justification of reading the series as culminating in a three trace, single gap condition was based upon the well defined tendency among the angiosperms toward a reduction in the amount of axial woody tissue. In all of the other genera except *Trollius*, a more or less complete similar series can be constructed; but in *Hepatica* (figs. 18, 17, 16), *Ranunculus*, *Anemonella*, and *Thalictrum* the series is especially complete. In *Hepatica*, at least, evidence exists of a further modification of the primitive condition. In this genus certain sepals show evidence that the three traces arising from a common gap have fused laterally to form a single strand (fig. 19). On the other hand, the single trace sepals of *Anemonella* and *Thalictrum* are probably due to a disappearance of the lateral traces; therefore the sepals of the Ranunculaceae are fundamentally three trace organs.

It is an interesting fact that among leaves, carpels, and sepals the tendency exists toward a reduction in the number of gaps formed

by the departing traces, and a fusion or a suppression of the lateral traces. This fundamental similarity of development illustrates another phase of plant evolution, namely, that all organs of one plant are not developing in the same direction at the same time. *Hepatica*, for instance, has the most highly evolved vascular supply to the carpel of the group, a more primitive sepal condition, and a foliar condition of another degree of specialization.

SUPPRESSED ORGANS.—The presence of vascular strands to suppressed organs, such as to suppressed ovules of *Trollius* and suppressed carpels of *Caltha*, demonstrates that an organ may disappear before the vascular supply to that organ disappears. On the other hand, the vascular supply does not always lag behind; its disappearance may precede that of the organ. Unpublished observations of the writer support this latter contention. In the sterile flower of *Sassafras varitifolium* (Salisb.) Ktze., a small globose pistil is present, but no vascular supply leads to it. Thus these observations upon the Ranales substantiate the statements that the vascular supply to an organ may disappear either before the organ, or after it. The third possibility (both disappearing at the same time) also occurs.

INTERRELATIONSHIPS.—Within the Ranunculaceae three or four major lines of divergence from the prototype have occurred. (1) *Caltha*, *Coptis*, *Trollius*, and *Actaea* have retained the multiovulate, three trace carpel, the spiral insertion of all parts, and the three trace sepal; they are therefore the least specialized group. (2) *Hepatica*, *Clematis*, *Anemone*, *Anemonella*, and *Ranunculus* have become specialized in that the three traces to each carpel have become fused to form a single strand, and in that the carpels are uniovulate. Within this group *Ranunculus* is the most specialized. The sepals are both whorled and five. Petals also have appeared, and are usually whorled and five in number. (3) *Thalictrum* possibly ought to be considered as a separate line of development because of its dioecious character; however it agrees with *Anemonella* in every other respect. (4) *Aquilegia* typifies the other distinct line of divergence. It has retained the multiovulate carpel receiving three distinct traces, but has become whorled as to the insertion of parts and has become arranged entirely upon the numerical plan of five. The presence of petals is also a specialized and advanced character.

Summary

As the descriptions, discussions, and general conclusions have shown, all of the 17 representative species of the Ranunculaceae studied agree as to fundamental floral vascular structure. The axis is a simple one. All floral organs are usually inserted spirally (modifications occur in *Aquilegia* and *Ranunculus*). Three types of floral organs are present: carpels, stamens, sepals. The sepals are fundamentally three trace trilacunar organs. The carpels also are fundamentally three trace trilacunar organs, and in addition are multiovulate. The uniovulate single trace carpel is derived from the former. This change has been accomplished by a gradual reduction in the number of ovules from many to few to one, and a lateral fusion of the three traces to form a single strand. Stamens are unique organs in that they always receive but a single trace. Petals are evidently modifications of stamens; hence they always differ from the sepals in being definitely single trace organs. Within this unified natural primitive family, three or four distinct lines of divergence occur: (1) *Caltha*, *Trollius*, *Coptis*, *Actaea*; (2) *Hepatica*, *Anemone*, *Clematis*, *Anemonella*, *Ranunculus*; (3) *Thalictrum*; (4) *Aquilegia*. Evidence exists also of the suppression of organs (carpels, *Caltha*; ovules, *Trollius*). Thus the aggregate of facts makes the low phylogenetic position of the Ranunculaceae more certain. This investigation also is forming the basis for another study. This is a comparative study of the vascular anatomy of the flowers of the Ranunculaceae with that of the remaining families of the Ranales and other groups considered to radiate from the "Ranalian plexus."

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MORPHOLOGY OF PREISSIA QUADRATA

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(WITH PLATES I, II AND SIXTEEN FIGURES)

Although the structural and developmental features of many of the Marchantiales have become familiar through a number of very thorough investigations, and as a result our knowledge of the group as a whole has been greatly enhanced, some of the commonest genera have received relatively little attention. One of these is *Preissia*, a form also known as *Chomiocarpon*.

There is no doubt as to the systematic position of this genus. The gross morphological characters, which are well known, render obvious its affinities to the other Marchantiales. SCHIFFNER (45) divides the Marchantiales into the Ricciaceae and Marchantiaceae, and the latter into the three subfamilies Corsinioideae, Targionioidae, and Marchantioideae. Following LEITGEB (38), he further splits up the Marchantioideae into the Astroporae, Operculatae, and Compositae. Later workers, such as CAVERS (10) and EVANS (31), consider these groups coordinate, raising them all to the rank of families. Thus EVANS separates the Marchantiales directly into the Ricciaceae, Corsiniaceae, Targioniaceae, Sauteriaceae (Astroporae), Rebouliaceae (Operculatae), and Marchantiaceae (Compositae). There seem to be valid reasons for this grouping; certainly it is more convenient than the older system. In the present paper the term Marchantiaceae is used in this restricted sense. CAVERS includes in the family the following eight genera: *Exormotheca*, *Conocephalum*, (*Fegatella*), *Lunularia*, *Wiesnerella*, *Dumortiera*, *Bucegia*, *Preissia*, and *Marchantia*. Thus *Preissia* constitutes one of the most highly specialized members of the order. It is generally recognized that its affinity to *Marchantia* is especially close.

It appears that *Preissia* is represented by but one species, widely distributed over the Northern Hemisphere. *P. quadrata* (Scop.) Nees is the name adopted for it by EVANS (31), *P. commutata* Nees, a name used by various other writers for the same species, being relegated

by him to synonymy. EVANS considers *P. quadrata* to be the sole representative of the genus in North America. He gives its distribution as Arctic America, Canada, and the northern United States as far south as Virginia and Colorado. The species also occurs in Europe and northern Asia. SCHIFFNER (45) claims that a second species occurs in Mexico, but does not give its name. It is almost certain that he refers to *P. mexicana* Stephani, a species which EVANS regards as identical with *Marchantia chenopoda* L.

Most of the material for the present study was collected in St. Lawrence County, New York, near the village of Canton. Collections were made during September and October of 1921 and 1922, and during April, May, and June of 1922 and 1923. Excellent material was also obtained near Fort Plain in Montgomery County on July 11, 1923. In both localities the plants were growing on thin soil covering granitic rocks, usually along stream banks. *Preissia* was found in relatively drier situations than *Marchantia* and *Conocephalum*, forms which occur in the same region.

Thallus

The gametophyte of *Preissia* consists of a dorsiventral, pale green thallus with somewhat wavy margins. It is thickened along the median line, on either side forming a thin lamella. Unlike *Marchantia*, it lacks a distinct midrib. The thallus branches dichotomously when young, but later forms apical innovations, in this respect also differing from *Marchantia*. The ventral surface bears two longitudinal rows of purple scales along the median line, and both smooth and pegged rhizoids. The scales are appendaged as in the other Marchantiaceae.

The main body of the thallus consists of uniformly compact parenchyma with a single layer of air chambers beneath the epidermis (fig. 1). These arise by intercellular cleavage, the split starting below the epidermis and becoming extended to the surface. The method of air chamber formation closely resembles that described by BARNES and LAND (2) for *Marchantia*. Chlorophyllose filaments arise from the floor and sides of the air chambers; they may be either simple or branched. The epidermis consists of a single layer of thin walled cells containing relatively few chloroplasts. The thallus air

pores are of the compound or barrel-shaped type found only in *Bucegia*, *Preissia*, and *Marchantia*. In the other genera of the Marchantiaceae and in the Rebouliaceae compound air pores are limited to

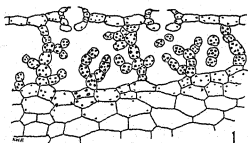


FIG. 1.—Upper region of thallus showing air chambers and pores; $\times 112$.

the receptacles. The pore opening is surrounded by 4-6 superimposed rings of cells, the lowest ring consisting of four large cells which project inward. GOEBEL (23) was the first to show that in *Preissia* these large cells are capable of causing a partial closure of the air pore when an excessive amount of water is lost

from the cells of the thallus. When the latter are fully turgid the air pores are wide open. The same behavior has been described by CAVERS (9). GOEBEL also found that the air pores of *Marchantia* do not close.

Chloroplasts are found in the cells just below the air chambers, but are absent from the cells farther down. Along the median line of the thallus the colorless region comprises more than half its thickness. Here the cells become more and more elongated toward the ventral side, the lower cells being 5-10 times as long as wide. The walls of these elongated cells are slightly thickened and bear pits. The occurrence of cells similarly pitted has been reported by Miss STARR (47) in *Plagiochasma*, and by BOLLETER (4) and CAVERS (8) in *Conocephalum*. In *Preissia*, however, the cells in the ventral region are more elongated than in the other forms.

As in many other liverworts, intracellular fungi live in the lower part of the thallus. They are present chiefly along the median line and are more abundant in the older parts of the thallus. The fungal zone often constitutes one-half to two-thirds of the thickness of the thallus, thus occasionally extending as far as the air chambers, but in most cases it is more limited. In the ventral region the hyphae are arranged in parallel strands extending longitudinally; above they form compact tangled masses inside the shorter cells of the thallus. The intracellular fungi of *Preissia* have been studied by GOLENKIN (26), CAVERS (7), and others.

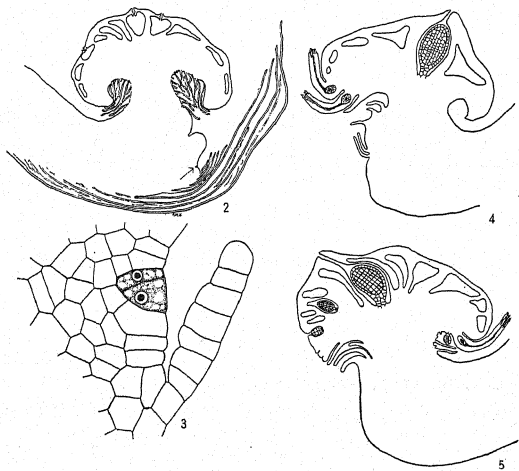
A notable feature of *Preissia* is the occurrence of scattered sclerotic cells in the ventral portion of the thallus. These were first described by GOEBEL (22). They are elongated, thick walled, dark brown, fiber-like cells with pointed ends. As GOEBEL reports, they ordinarily occur singly, but occasionally in cross-section two or three may be seen together. Their significance is uncertain. CAVERS (9) thinks that their chief function is the conduction and storage of water, pointing out that no fibers occur in plants grown in a moist atmosphere. Isolated mucilage cells are present in all parts of the thallus, but mucilage canals are absent. Starch grains do not accumulate conspicuously except in the older female receptacles, where the presence of reserve food is obviously related to the growth of the sporophytes. Oil globules are present in the apical region, especially in the epidermal cells, and in the epidermis of the receptacles.

Growth of the thallus is by means of a single cuneate apical cell which cuts off segments from its four sides (fig. 3). This is the prevailing type among the Marchantiales. The apical cell is small and not easily seen in sections through the growing notch. In most of the higher Marchantiales the formation of a receptacle terminates continued dichotomy of the growing apex. This condition prevails in *Preissia*, but here further growth of the thallus occurs through the formation of apical innovations ("ventral adventitious shoots"). Soon after a receptacle begins to form, the new shoot arises beneath it as a small rounded elevation. Below is situated the apical cell which has produced this primordium, and which continues its growth (figs. 2, 3). Apical innovations may arise beneath both male and female receptacles. The growing apex gives rise to a dorsal branch, which by further forking forms the receptacle, and a ventral branch which forms the apical innovation. The latter grows as the receptacle is developing, and later produces a new receptacle in its apical notch. In regard to the origin of the adventitious branches in *Targionia*, DEUTSCH (13) says that they "have absolutely nothing to do with the apical cell."

Receptacles

For the most part, the receptacles of *Preissia* are unisexual and dioecious. Only a few monoecious plants were seen, about 1 per cent in the material studied. A great many bisexual receptacles were

found, however, as will appear later. In *Preissia* both male and female receptacles are stalked. The occurrence of stalked male receptacles is an advanced feature, characteristic only of *Dumortiera*, *Bucegia*, *Preissia*, and *Marchantia*. Both kinds of receptacles repre-



FIGS. 2-5.—Fig. 2, median longitudinal section of thallus with female receptacle showing position of apical cell and origin of apical innovation, $\times 60$; fig. 3, enlargement of fig. 2 showing apical cell cutting off dorsal segment, $\times 665$; figs. 4, 5, young bisexual receptacles; $\times 75$.

sent a definite branch system, terminating dichotomous apical growth of the thallus by the original apical cell, as previously explained. The stalk of the female receptacle does not begin to elongate until the sporophytes are nearly mature. In this respect *Preissia* shows a marked contrast to *Marchantia*, resembling *Asterella*, *Reboulia*, *Conocephalum*, etc.

The antheridia first appear in the late spring, the archegonia in the early summer. Sex organs continue to be formed during the

whole growing season, young archegonial receptacles appearing as late as the last of September in northern New York. The archegonial receptacles live over the winter with the sporophytes in the stage in which the sporogenous tissue has just been differentiated, as also noted by Miss GRAHAM (27). Sometimes, however, the development goes farther before winter. Growth of the sporophytes and of the receptacle stalk continues in the spring, the spores maturing in June.

Both kinds of receptacles have compound air pores like those found on the thallus, and air chambers with green filaments. In both cases the receptacle stalk has two rhizoid furrows and is without green tissue. The air chambers of the male receptacle are deep and narrow, while those of the female receptacle and thallus are alike. There are few or no chlorophyllose filaments in the deeper air chambers in the center of the antheridial disk between the pits containing mature antheridia, but only in the superficial portion of the disk. CAVERS (9) says that green filaments are absent from the margin of the receptacle, but this was not found to be the case. The male receptacles are disklike and slightly convex above with a thin margin. They are but slightly or not at all lobed. EVANS (31) notes that the antheridia occur mostly in four, sometimes 3-6 radiating rows formed in acropetal succession. The antheridial chamber opens to the top of the disk by a simple type of pore, the pore margin consisting of unthickened cells but slightly raised above the surface. In no way is the pore modified.

The female receptacles are hemispheric, with mostly four inconspicuous lobes and four prominent ridges alternating with them. These ridges are to be regarded as homologous with the conspicuously developed rays of the female receptacle of *Marchantia*. The cavity on the under side of each ridge contains a group of hairlike paraphyses (fig. 2). The archegonia arise beneath the lobes, each group being inclosed by a single membranaceous involucre having an entire margin. LEITGE (38) has pointed out that the archegonia show a tendency toward a tangential arrangement, instead of being arranged radially as in *Marchantia*. Ordinarily about four or five archegonia are formed in each group. In this respect *Preissia* stands midway between such forms as *Reboulia* and *Conocephalum*, where normally only one archegonium occurs in each notch, and *Mar-*

chantia, where many are formed. In the material studied only one sporophyte develops under each lobe as a rule, although in rare cases two were seen under the same lobe. CAVERS (9) states that in most cases two sporophytes mature in each archegonial group.

Bisexual receptacles

✓The material collected at Fort Plain, as it occurred in the field, consisted mostly of plants bearing young receptacles having the general male form, a few plants (about 5 per cent of the total) with nearly mature male receptacles, and a very few (less than 5 per cent) with very young female receptacles. Upon sectioning the youngest male receptacles, it was found that about half of them bore both antheridia and archegonia. It was estimated that these bisexual receptacles comprised approximately 30 per cent of the total collection. It is noteworthy that none except the very youngest receptacles were bisexual.

The Canton material, collected at intervals from the middle of September to the middle of October, consisted mostly of plants bearing receptacles having the general female form. About 20 per cent of these were bisexual. There were a few old strictly antheridial receptacles, but no young ones. Still fewer old receptacles were found having elongated stalks and the typical male form, but bearing one or two (rarely three) nearly mature sporophytes. These facts indicate that the greatest proportion of strictly antheridial receptacles appears at the beginning of the season, and the greatest proportion of strictly archegonial ones at the end. It is also apparent that the greatest proportion of bisexual receptacles is found at the middle of the growing season, their number increasing to a maximum and then decreasing.

✓Bisexual receptacles in the Marchantiales were first reported by TAYLOR (49), who found them in *Dumortiera irrigua*. In a later paper (50) the following statement is made with reference to this species: "The fructification is commonly dioecious, sometimes monoecious, and not rarely androgynous as observed in *Marchantia androgyna*." The latter is an old name for *Preissia quadrata*. In TAYLOR's earlier paper *Preissia* is mentioned, but nothing is said of its being androgynous. ✓

✓GOEBEL (21) later studied bisexual receptacles in *Preissia*, noting that the antheridia are borne on the upper side of the anterior portion of the receptacle, and the archegonia on the under side of the posterior portion. Because the receptacles are typically unisexual, he considers that either part of a male receptacle has produced archegonial primordia which have then undergone a displacement to the lower side, or vice versa. He regards this as a mere matter of displacement which does not represent a reversion to a more primitive distribution of sex organs.✓

✓LEITGEB's study (38) of this situation in *Preissia* differs in several important respects from that of GOEBEL. He found a large clump of plants which bore bisexual receptacles exclusively, and when grown in culture did the same thing the next year. Without exception the anterior half of each receptacle was female and the posterior half male, whereas GOEBEL had found just the opposite relation to exist. The forward portion bore two archegonial groups which corresponded in every way to the two anterior groups of a normal female receptacle, while the posterior half bore on its dorsal surface the sunken antheridia. As on a normal male receptacle, the male part of the disk had a thin margin, but this turned down on either side as it went forward to the archegonial groups. LEITGEB considers that, in the case of the bisexual receptacles, the differentiation of sex is delayed until the branches of the receptacle are formed.✓

ERNST (17, 18) finds that bisexual receptacles occur abundantly in *Dumortiera trichocephala* and exceptionally in *D. velutina*. The arrangement of the sex organs does not coincide with that found in *Preissia* by either GOEBEL or LEITGEB, as here on one side is seen the typical configuration of the female receptacle with archegonia, and on the other side the regular form of the male receptacle with antheridia in pits. CAMPBELL (6) confirms the occurrence of bisexual receptacles in *D. trichocephala*. CUTTING (11) observed a few cases in *Marchantia* where a female receptacle with functional archegonia produced an antheridium-bearing lobe as a proliferation from its under side. He correctly regards such cases as abnormalities and not comparable with the bisexual receptacles of *Preissia*. In GOEBEL's study of *Monoselenium* (25) some bisexual receptacles were found with archegonia in front and antheridia behind. In his study of *Re-*

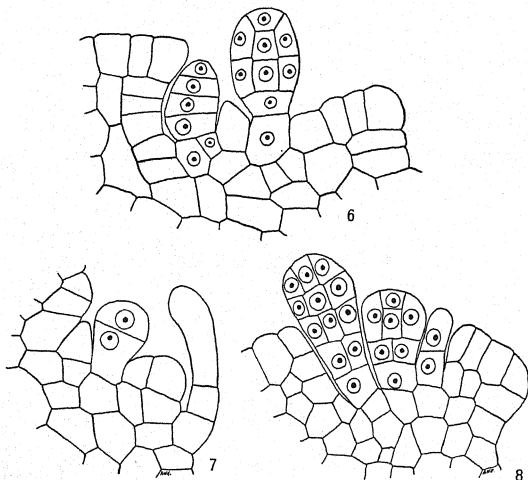
boulia, DUPLER (14) found a few female receptacles bearing male sex organs, and vice versa.

When first formed, the receptacles of *Preissia* are small rounded elevations indistinguishable from one another until the first sex organs are formed. If antheridia are first produced on a receptacle, as ordinarily happens during the early and middle part of the summer, the general form of the receptacle comes to be male, that is, the top becomes flat, the margin thin, and the air chambers deep and narrow. If sooner or later, while the receptacle is growing, archegonia arise at one or more of the growing points, a lobe forms above them, a lobe such as characterizes a typical female receptacle. This portion of the receptacle then forms shallow air chambers and becomes otherwise distinctively female. If archegonia arise on an antheridial receptacle at all, they do so while it is still relatively young. It should be understood that after a growing point has once produced archegonia, it never gives rise to antheridia again. Thus if archegonia first arise on a young receptacle at all four growing points, the receptacle assumes the typical female form. Several cases were observed in which young archegonia were arising immediately in front of young antheridia. One of the most interesting is shown by figs. 6, 7. This demonstrates conclusively that both kinds of sex organs may arise as segments of the same apical cell, and that the formation of archegonia immediately follows the production of antheridia.

In most of the bisexual receptacles examined, either both anterior groups of sex organs were of one sex and both posterior groups of the other sex, or less frequently three were of one sex and one of the other. In a few cases both of the groups on one side of the receptacle were of the same sex, and both groups on the opposite side of the other sex. The latter is similar to the arrangement ERNST found in *Dumortiera*. In no case, however, were groups diagonally opposite each other on the receptacle found to be of the same sex. In most of the preparations of young bisexual receptacles studied, and in many of the older ones, one or both of the anterior lobes were producing archegonia while antheridia were confined to the central and posterior portions of the receptacle (fig. 4). This is similar to the condition seen by LEITGEB. A few young bisexual receptacles were examined,

and some older ones, in which archegonia were being produced only under one or both of the posterior lobes (fig. 5). This coincides with the situation described by GOEBEL.

The present study of bisexual receptacles in *Preissia* justifies the



FIGS. 6-8.—Young archegonia arising on bisexual receptacle immediately in front of young antheridium; $\times 665$; fig. 7 drawn from next section; fig. 8, young sex organs arising on bisexual receptacle: to left a transitional type, in center a young archegonium, to right an archegonium initial; $\times 665$.

following general conclusions. (1) It seems well established that there is a definite time relation involved in the appearance of sex organs. (2) It appears certain that all of the plants contain the possibilities of producing either kind of sex organs. Although the term "dioecious" is used to describe the usual condition of sexuality in *Preissia*, sexual differentiation of thalli does not exist. (3) The general form of the receptacle is determined by the kind and number of

sex organs it produces. The receptacle of *Preissia* is a plastic structure, undifferentiated at first, and capable of assuming either male or female characters. (4) This is strongly suggestive of a common origin, phylogenetically, for the highly differentiated male and female receptacles of the higher Marchantiales. (5) As to what determines whether antheridia or archegonia shall be produced on a given receptacle, this investigation throws no light. (6) The significance of the occurrence of bisexual receptacles in *Preissia* is uncertain.

Antheridium

The antheridia arise in acropetal succession from segments of the apical cell. Their development corresponds to the regular Marchantiales type. The superficial papillate initial (fig. 17) divides transversely (fig. 18), the basal cell forming the imbedded portion of the stalk and the outer cell the rest of the antheridium. A filament of four cells is then formed from the latter by the appearance of three additional transverse walls (figs. 19-21), thus agreeing with STRASBURGER's account (48) of *Marchantia*. The sequence of these walls was not apparent. The writer (29) has shown that in *Reboulia* there seems to be no definite sequence; at least there is not the centrifugal succession of cell walls which STRASBURGER claims for *Marchantia*. According to DURAND (16), the superficial portion of the antheridium of *Marchantia* at this stage probably consists of but three cells. He observed no young antheridium with more than two transverse walls in its superficial portion. ABRAMS (1) found the same situation in *Cryptomitrium*.

Vertical walls are now formed in two planes at right angles to each other (figs. 22-24). These commonly appear first in the basal tiers, but often elsewhere, as reported by DURAND (16) for *Marchantia* and by the writer (29) for *Reboulia*. In the latter the appearance of the first vertical walls immediately following the formation of four tiers of cells in the superficial portion of the antheridium has been shown to be a constant feature. Furthermore, both DURAND and the writer find that following the appearance of the first vertical walls, additional transverse walls may come in, resulting in the formation of five or six tiers of cells. This may also take place in *Preissia* (figs. 25, 26).

In a recent study of *Reboulia*, DUPLER (14) confirms the development of the antheridium described by the writer, but finds that occasionally four transverse walls may be formed in the upper cell before the vertical walls appear. CAMPBELL (5) reports a similar condition in *Asterella*. BOLLETER (4) finds that in *Conocephalum* three or four transverse walls appear as a rule, but that the young antheridium may consist of as many as eight superimposed cells before the vertical walls come in. It is likely that this may happen in other Marchantiales, but was not observed in the present study.

As in *Reboulia*, the formation of periclinal walls cutting off the sterile jacket from the inner spermatogenous cells involves only the three uppermost tiers, the lower cells contributing to the stalk (fig. 27). This is apparent even in older antheridia, because the original division walls often stain more heavily than the later ones (fig. 28). Further growth of the spermatogenous tissue is typical, and need not be described.

CAMPBELL (5) states that in *Riccia* "the lower one or two segments and the terminal ones do not take part in the formation of sperm cells, but simply form part of the wall of the antheridium." ABRAMS (1) finds the same unusual situation in *Cryptomitrium*. He states that the spermatogenous tissue is derived exclusively from the middle of the three original cells into which the superficial portion of the young antheridium is divided, the upper and lower cell taking no part but contributing to the antheridium wall. Miss McFADDEN (41) reports a similar condition in *Targonia*. This behavior is not in agreement with any other investigation on the Marchantiales. That the upper cell of the young antheridium does contribute to the spermatogenous tissue is strongly suggested by a careful examination of consecutive stages in both *Reboulia* and *Preissia*, but the critical mitotic figure to establish the point was not found in these forms. Among the writer's preparations of *Marchantia polymorpha*, however, an antheridium was found which definitely proves the case (fig. 29).

Archegonium

The archegonial receptacle arises at the growing point of the thallus as a small rounded knob, and, as in all of the higher Marchantiales, the apical cell is involved in its formation. A new apical cell forms in each receptacle notch, of which in *Preissia* there are four.

The archegonium initials do not appear until the young receptacle is conspicuously dome-shaped. The superficial papillate initial (fig. 32) undergoes a transverse division, forming a basal and an outer cell (fig. 7). The latter gives rise to the primary axial cell and the primary wall cells, by the development of three vertical walls in the way characteristic of all Bryophytes (figs. 33-34). At the same time the basal cell commonly divides by a transverse wall.

This sequence of wall formation (except that in some cases the basal cell does not divide) has been reported by JANCZEWSKI (33) for *Riccia*, by CAMPBELL (5) for *Riccia*, *Targionia*, and *Asterella*, by GARBER (20) and LEWIS (39) for *Ricciocarpus*, by LANG (37) for *Cyatodium*, by ABRAMS (1) for *Cryptomitrium*, by Miss STARR (47) for *Plagiochasma*, by HAUPT (29) for *Reboulia*, by CAVERS (8) for *Conocephalum*, and by DURAND (16) for *Marchantia*. STRASBURGER (48) states that in *Marchantia* the outer cell undergoes a transverse division before the three vertical walls appear. The same condition has been reported by JANCZEWSKI (33) for *Preissia* and *Lunularia*, and by BOLLETER (4) for *Conocephalum*. In none of these cases, however, are mitotic figures shown as proof, and hence the orthodox interpretation is possible.

Further development of the archegonium of *Preissia* is in agreement with the usual Marchantiales situation (figs. 34-39). After the primary neck canal cell has given rise to four neck canal cells by two successive divisions (figs. 37, 38), the primary ventral cell forms the ventral canal cell and egg (fig. 39), as previously reported for *Preissia* by JANCZEWSKI (33). The first division of the primary neck canal cell is accompanied by the formation of a vertical wall in the cover cell (fig. 37). Miss STARR (47) has shown that in *Plagiochasma* eight neck canal cells are formed before the primary ventral cell divides. In *Reboulia* (29) the situation is similar to that in *Preissia*, but the number of neck canal cells is later increased to 18-20. CAVERS' (8) figures show that in *Conocephalum* the primary ventral cell divides after only two cells have been formed from the primary neck canal cell, while BOLLETER'S (4) figures show that the development is normal. Both JANCZEWSKI (33) and DURAND (16) state that in *Marchantia* the primary ventral cell may occasionally divide prematurely.

The stalk of the mature archegonium is short. The neck curves

outward and upward, as in other forms where the receptacle stalk does not elongate until the sporophytes are approaching maturity. The mature archegonium of *Preissia* invariably contains four neck canal cells (fig. 39). It may also be stated with certainty that this number is not increased by later divisions, as in *Reboulia*. As in the other Marchantiales, the neck shows six cells in cross-section.

The range in variation among the Marchantiales in regard to the number of neck canal cells is shown in table I. It will be noted that the prevailing number is four, although some forms show the more primitive number eight. *Reboulia* is the only form credited with a larger number.

TABLE I
NUMBER OF NECK CANAL CELLS IN MARCHANTIALES

GENUS	WRITER	NUMBER REPORTED
Riccia.....	Janczewski, Campbell	4
Ricciocarpus.....	Garber, Lewis	4
Cyathodium.....	Lang	8-9
Plagiochasma.....	Janczewski	4
Plagiochasma.....	Starr	8
Cryptomitrium.....	Abrams	8
Reboulia.....	Janczewski	4
Reboulia.....	Haupt	18-20
Conocephalum.....	Cavers, Bolleter	8
Lunularia.....	Janczewski	4
Preissia.....	Janczewski, Haupt	4
Marchantia.....	Janczewski, Durand*	4

* Sometimes six or seven nuclei.

✓ Anomalous sex organs

In a form having bisexual receptacles, like *Preissia*, the finding of occasional aberrant sex organs might be expected. That they have been reported in other Bryophytes is a matter of common knowledge. A few interesting cases were seen during the course of the present investigation which will be described briefly. As already pointed out, ✓ there is a definite time relation in the appearance of sex organs on the bisexual receptacles, the antheridia always developing first. It has been seen that in some cases the young archegonia and antheridia arise side by side (fig. 6). Ordinarily these are easily recognizable as either male or female organs, but sometimes young sex organs are seen which do not correspond to either type. It is impossible to say

into what kind of organ these aberrant forms might have developed. Presumably they would eventually have produced either sperms or eggs, but this could not be determined at their present stage of development. Thus in fig. 8 the oldest organ might be either an antheridium or an archegonium. The middle one is unquestionably an archegonium, and the youngest an archegonium initial. This particular receptacle had already borne antheridia. Thus the oldest organ represents a transitional type.

Fig. 40 represents an organ produced on a male receptacle. It shows no resemblance to an archegonium, and yet it is very unlike a typical young antheridium. It probably would have developed into an antheridium of an unusual kind. Just to one side of this organ, nearer the center of the disk, was seen a peculiar antheridium (fig. 41) with a restricted group of spermatogenous cells; its upper portion was prolonged into a neck which projected slightly out of the antheridial chamber.

Fig. 42 is a strictly median longitudinal section through an organ found on a female receptacle. It was borne in the position of an archegonium, yet had somewhat the appearance of an antheridium. If it were to be a normal antheridium, it should be sunken in its pit by this time. It would be interesting to determine, were it possible to do so, whether this organ would produce sperms or an egg.

The most interesting anomaly is shown by fig. 31. Here is an organ developed in a typical antheridial chamber, with a stalk half its length, and with an axial row of three cells surrounded by a sterile jacket. From a careful examination of the preceding and following section, it may be said that the sterile jacket consists of either five or six rows of cells. This organ seems to be an antheridium developing as an archegonium, a truly transitional form. The axial row consists of protoplasm which stains more densely than the other cells; in fact, it has every appearance of a young archegonium as represented by fig. 37, with the exception of its very long stalk and position in an antheridial pit. Just what was the appearance of this organ at an earlier stage of development can only be conjectured. Had development proceeded, it seems very likely that it would have become an egg producing organ.

Based on studies both in the Bryophyta and Pteridophyta, a

great deal of evidence has accumulated in support of the view that phylogenetically the canal cells are eggs which have ceased to function, and that the antheridium and archegonium are homologous organs, the spermatogenous tissue of the former corresponding to the entire axial row of the latter. This evidence has been derived chiefly from a study of abnormalities in development. The subject is well discussed in papers by GOEBEL (24), DAVIS (12), HOLFERTY (32), MISS LYON (40), MEYER (43), KURSSANOW (36), FLORIN (19), and others. Striking evidence that the antheridium and archegonium have had a common origin is seen in the occurrence of bisexual organs described by HOLFERTY, MEYER, and others, where the same gametangium may produce both eggs and sperms. The present study of *Preissia* adds evidence of a new sort to the view that the two kinds of sex organs of Bryophytes are homologous.✓

Fertilization

A great many cases of fertilization were observed in material collected September 24, 1921, at Canton, New York. Practically all of these were in the same condition, the male nucleus within the egg and in contact with the female nucleus, but not yet fused with it. This stage has been observed by Miss BLACK (3) in *Riccia*, by GARBNER (20) in *Ricciocarpus*, by MEYER (42) in *Corsinia*, and by WOODBURN (51) and DUPLER (15) in *Reboulia*. Judging from the frequency of this stage in my preparations, it seems very likely that the sperm and egg nuclei do not fuse immediately upon coming together, but remain in contact for a while.

Before the entrance of the sperm, the chromatin of the egg nucleus surrounds the nucleolus as a dense mass (fig. 39). Only two cases were observed in which the male and female nuclei had not yet come into contact. In both instances the chromatin of the egg nucleus was in the form of a reticulum (fig. 43). Of the many cases seen in which the sexual nuclei were in actual contact, the chromatin of both nuclei was reticulate in only four cases (fig. 44), while in twenty-one cases in both egg and sperm it was contracted into a dense mass around the nucleolus (fig. 45). It seems likely that the latter represents the more advanced condition of development.

Miss GRAHAM (28) has described and figured centrosomes in

Preissia at the time when the male and female nuclei come together. She saw centrosomes with astral rays at either pole of the egg nucleus. After a critical examination of all available fertilization stages, the writer demonstrated to his own satisfaction the presence of centrosomes such as Miss GRAHAM describes in about ten cases out of forty (fig. 45). In some instances, as in fig. 43, the centrosomes were above and below the egg nucleus, thus appearing in the preceding and following section. Miss GRAHAM speaks of the egg cytoplasm as consisting of an inner granular zone and an outer coarsely vacuolar zone. It seems to the writer that her use of the term "zones" is forced. A greater concentration of cytoplasm around the nucleus is perfectly apparent, but it does not constitute a definitely delimited portion of the cell such as the term "zone" implies.

In many cases the cytoplasm is particularly dense between the fusing nuclei (figs. 43-44). In regard to this matter Miss GRAHAM states:

Between the antherozoid and the egg nucleus lies a small mass or body unlike the cytoplasm just described. From its position in close proximity to the nucleus of the antherozoid, the small quantity visible, and the absence of similar cytoplasm anywhere else in the cell, it might be thought to be cytoplasm brought in by the antherozoid, but I have no proof that this is the case.

Miss GRAHAM does not explain why there should be so much of this male cytoplasm and why it should be pushed ahead of the sperm nucleus. Her interpretation on this basis of the presence of dense cytoplasm at a place of high metabolic activity seems to be entirely without evidence.

Chromosome number

Although a great many mitotic figures were observed in the preparations, in only a very few cases could the chromosomes be counted. Miss GRAHAM (27) made a very thorough study of mitosis in *Preissia* without discovering the chromosome number. She states that it is probably eight, but does not give convincing evidence. The chromosomes in the vegetative cells are described and figured as small oval bodies, as seen in both side and polar view. In her study no centrosomes were seen.

A number of particularly clear polar views of mitotic figures were seen in a half-grown antheridium occurring with others on a

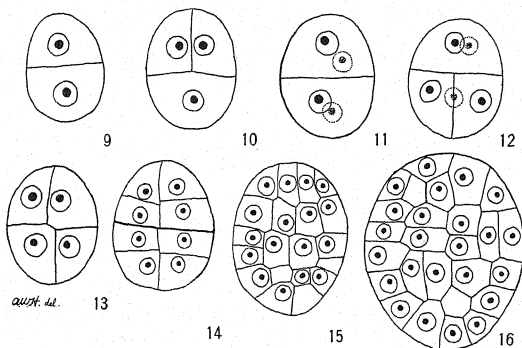
bisexual receptacle. The chromosomes were entirely separate from one another and sharply stained. The haploid number is unquestionably nine (fig. 30). One chromosome is much smaller than the others, being nearly spherical. The others are five to ten times as long as wide. Unfortunately, cases favorable for counting were not found on other preparations; hence it cannot be stated whether or not there is any relation between the sexual condition of a given plant and its chromosome equipment.

SHOWALTER (46), studying *Conocephalum conicum*, a dioecious species, found no visible differences between the sexes. He demonstrated that the chromosome number is nine instead of eight, as reported by earlier workers, and that one of the chromosomes is much smaller than the others. *Conocephalum* and *Preissia* are therefore alike in this respect. Chromosome numbers have not been determined in a large number of the Marchantiales. Miss BLACK (3) reports eight for *Riccia Frostii*, LEWIS (39) and GARBNER (20) four for *Ricciocarpus*, and MEYER (42) eleven for *Corsinia*. The numbers given are for the gametophyte.

Embryo

The first wall to be formed in the fertilized egg is transverse (fig. 9), or sometimes slightly oblique, and results in two approximately equal segments. Each cell then divides by a vertical wall, and later by another vertical wall at right angles to the first one, resulting in the formation of an octant (figs. 10-13). This behavior has been reported by KIENITZ-GERLOFF (34) for *Riccia*, by CAMPBELL (5) for *Riccia*, *Targionia*, and *Asterella*, by MEYER (42) for *Corsinia*, by LANG (37) for *Cyathodium foetidissimum*, by ABRAMS (1) for *Cryptomitrium*, and by KIENITZ-GERLOFF (34) and DURAND (16) for *Marchantia*. KIENITZ-GERLOFF (34, 35) states that the same type of development prevails in *Preissia* and possibly in *Grimaldia*, but his figures do not show the earliest stages. Further divisions occur in both the hypobasal and epibasal portions of the embryo, resulting in a more or less globular mass of cells (figs. 14-16). It has been shown by KIENITZ-GERLOFF (35) in *Preissia* and by DURAND (16) in *Marchantia* that the capsule is derived from the epibasal cell, and the foot and seta from the hypobasal.

As demonstrated by WOODBURN (51), HAUPT (30), and DUPLER (15), the embryo of *Reboulia* develops without the formation of an octant stage, the first three walls being transverse. BOLLETER (4) finds a similar condition in *Conocephalum*. LANG (37) finds a quadrant in *Cyathodium foetidissimum*, and a filament of four cells in *C. cavernarum*. Although CAMPBELL (5) finds an octant stage in *Targionia hypophylla*, Miss O'KEEFFE (44) states, "No such stage was observed, all the young embryos seen consisting of a single row of as many as five cells, but more often either three or four, before any longitudinal



FIGS. 9-16.—Stages in development of embryo; $\times 450$

divisions had taken place." Miss STARR (47) figures just one embryo of *Plagiochasma*, which indicates that its development resembles that of *Reboulia*. In *Dumortiera*, CAMPBELL (6) finds that the first two walls are transverse, and that "it is not unlikely" that another transverse wall may appear before the coming in of the vertical walls. In *Ricciocarpus*, GARBER (20) finds that the young embryo may consist of either a quadrant or a row of four cells. In *Conocephalum*, CAVERS (8) reports that the first wall is transverse. Sometimes a second transverse wall appears in the epibasal cell, but usually the next walls are vertical, dividing the embryo into an octant.

DUPLER (15) claims that occasionally a functioning triangular apical cell may be formed in the young embryo of *Reboulia*, the em-

bryogeny thus showing "an occasional partial agreement with that characteristic of the Musci." He also finds a triangular cell at the base of the embryo in some cases. This had earlier been reported by WOODBURN (51) and disputed by HAUPT (30). Because the vertical axis of the elongated embryo of *Reboulia* is often not straight but slightly curved, and because any of the walls may be somewhat inclined (not always perpendicular to the others), sections often reveal the presence of a triangular cell at either end of the embryo. Thus it appears when an anticlinal wall intersects one of the first vertical walls if either be slightly curved.

The writer has observed the same thing in the present study. It is also shown by the figures of other investigators, such as DURAND's (16) figs. 84 and 86, KIENITZ-GERLOFF's (35) figs. 34 and 6, and MEYER's (42) figs. 6, 7, 8, etc., but none of these authors claims that a "functioning apical cell" exists. Concerning the early embryo of *Targionia*, CAMPBELL (5) writes as follows:

In no cases seen was there any indication of a two-sided apical cell such as HOFMEISTER figures for *Targionia*, and probably his error arose from a study of forms where the quadrant walls were somewhat inclined, in which case the intersection of one of the secondary walls with it might cause the apex of the embryo to be occupied by a cell that, in section, would appear like the two-sided apical cell of the moss embryo.

KIENITZ-GERLOFF (35) finds in both *Preissia* and *Grimaldia* that the triangular cell often formed at the apex of the young embryo is not an apical cell. Regarding the embryo of *Preissia*, he writes as follows:

Die betreffenden Querwände verlaufen indessen selten horizontal, meist schliessen auch sie mit der Verticalen einen mehr oder weniger spitzen Winkel ein und so erhält man häufig, sowohl auf Aussenansichten, wie auf Längsschnitten am Scheitel des Embryo das Bild einer durch zwei entgegengesetzt geneigte Wände eingeschlossenen Scheitelzelle, ein Bild, das noch täuschender wird, wenn sich an die letzt entstandene, oberste Querwand wiederum eine zum Scheitel verlaufende, tangential ansetzt. Es hat in diesem Falle ganz den Anschein, als ob der Embryo in der That mit zweischneidiger Scheitelzelle wüchse (Taf. IX. fig. 6), und Bilder dieser Art sind es offenbar gewesen, welche Hofmeister veranlassten, für den Marchantiaceen-Embryo ein derartiges Wachstum anzunehmen. Dass ein solches nicht Statt hat, das lehrt uns einerseits die meist noch an älteren Stadien durch ihre bedeutende Stärke hervortretende Quadranten- und Octantenwand, andererseits jede Drehung des Embryo um seine Längsaxe.

Older sporophyte

The later development and mature characteristics of the sporophyte of *Preissia* present no striking features. The mature capsule is spherical, the seta relatively short, and the foot bulbous. The capsule wall is composed of a single layer of cells except at the apex. There a cap is present three or four cells thick in the middle. Annular bands are present in the capsule wall. CAVERS (9) has shown that a few fixed elaters are attached to the apical cap, and that fixed elaters may also arise from the base of the capsule. As in *Marchantia*, a perianth is formed around the sporophyte.

Summary

1. *Preissia* comprises a single species, *P. quadrata*, closely related to *Marchantia*.

2. The thallus is dichotomous when young, later forming apical innovations. It lacks a distinct midrib. Both smooth and pegged rhizoids and appendaged scales are borne below.

3. There is a single layer of air chambers with green filaments; their origin is schizogenous. The epidermal cells are thin walled and contain few chloroplasts. All air pores are barrel-shaped.

4. The colorless ventral cells are elongated and have thickened pitted walls. Scattered sclerotic cells occur in the ventral region.

5. Growth is by means of a single cuneate apical cell. After a receptacle has arisen, another apical cell forms an apical innovation which continues growth of the thallus.

6. The receptacles are mostly unisexual and dioecious, rarely monoecious, but very commonly bisexual. Both male and female receptacles are stalked, and both represent a branch system. Elongation of the female receptacle stalk is delayed.

7. Both receptacles have four growing points as a rule, the male showing greater variability in this respect than the female. The female receptacle has four inconspicuous lobes and four prominent ridges, the latter being incipient rays. Ordinarily about four or five archegonia are formed in each of the four groups.

8. A greater proportion of antheridial receptacles appears during the early part of the growing season, and of archegonial ones dur-

ing the latter part. The greatest proportion of bisexual receptacles occurs during the middle of the season.

9. On bisexual receptacles the production of antheridia always precedes the formation of archegonia.

10. All of the thalli contain the potentialities of either sex.

11. The general form of a receptacle is determined by the kind and number of sex organs it produces.

12. The antheridia develop like those of the other Marchantiales. The formation of periclinal walls delimiting the spermatogenous cells involves the three uppermost tiers of cells of the young antheridium.

13. Early development of the archegonium is typical. The ventral canal cell and egg are differentiated after four neck canal cells are formed; this number is not later increased.

14. Several anomalous sex organs are described, giving evidence as to a common origin phylogenetically for the antheridium and archegonium.

15. At the time of fertilization, at least in some cases, there is a centrosome with astral rays at opposite poles of the egg nucleus. The condition of the nucleus and cytoplasm at this time is described.

16. The haploid number of chromosomes is nine, one being very small.

17. The embryo develops with the octant stage characteristic of certain related genera. A functioning apical cell does not occur in the embryo of the Marchantiales.

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EXPLANATION OF PLATES I, II

All figures except fig. 29 are of *Preissia quadrata*.

FIGS. 17-28.—Stages in development of antheridium.

FIG. 17.—Antheridium initial; $\times 665$.

FIG. 18.—First division of same into basal and outer cells; $\times 665$.

FIGS. 19-21.—Division of outer cell to form filament of four cells; $\times 665$.

FIGS. 22-24.—Formation of vertical walls; $\times 665$.

FIGS. 25, 26.—Appearance of additional transverse walls; $\times 665$.

FIG. 27.—Completion of periclinal wall formation; $\times 665$.

FIG. 28.—Slightly older stage; $\times 665$.

FIG. 29.—Young antheridium of *Marchantia polymorpha* showing formation of periclinal walls in upper cells; $\times 665$.

FIG. 30.—Cells from half mature antheridium showing eight large chromosomes and one small one; $\times 3000$.

FIG. 31.—Sex organ showing both male and female characters; $\times 665$.

FIGS. 32-39.—Stages in development of archegonium.

FIG. 32.—Archegonium initial; $\times 665$.

FIG. 33.—Formation of primary wall cells and division of basal cell; $\times 665$.

FIGS. 34, 35.—Formation of cover cell and central cell from primary axial cell; $\times 665$.

FIG. 36.—Formation of primary neck canal cell and ventral cell from central cell; $\times 665$.

FIGS. 37, 38.—Division of primary neck canal cell to form four neck canal cells, and formation of vertical wall in cover cell; $\times 665$.

FIG. 39.—Mature archegonium showing differentiation of ventral canal cell and egg from ventral cell; $\times 665$.

FIG. 40.—Young sex organ developed on male receptacle; $\times 665$.

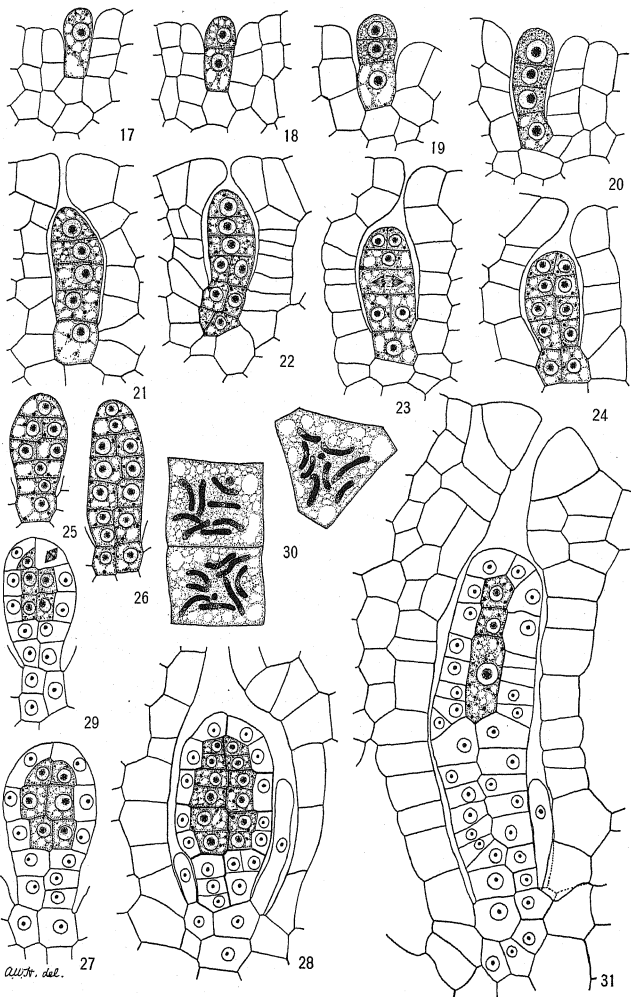
FIG. 41.—Older sex organ from same receptacle; $\times 665$.

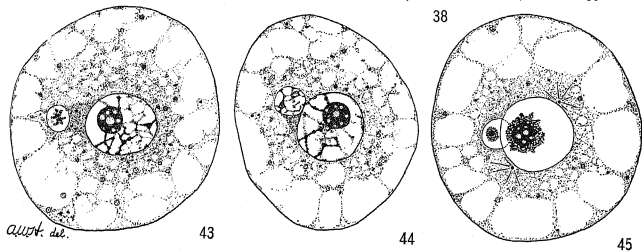
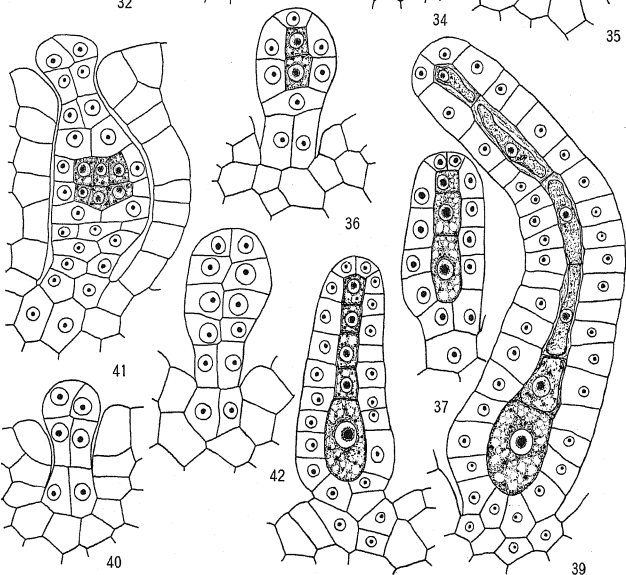
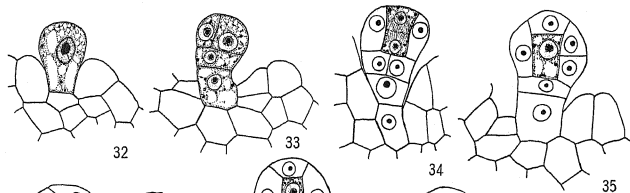
FIG. 42.—Median section of sex organ formed on female receptacle; $\times 665$.

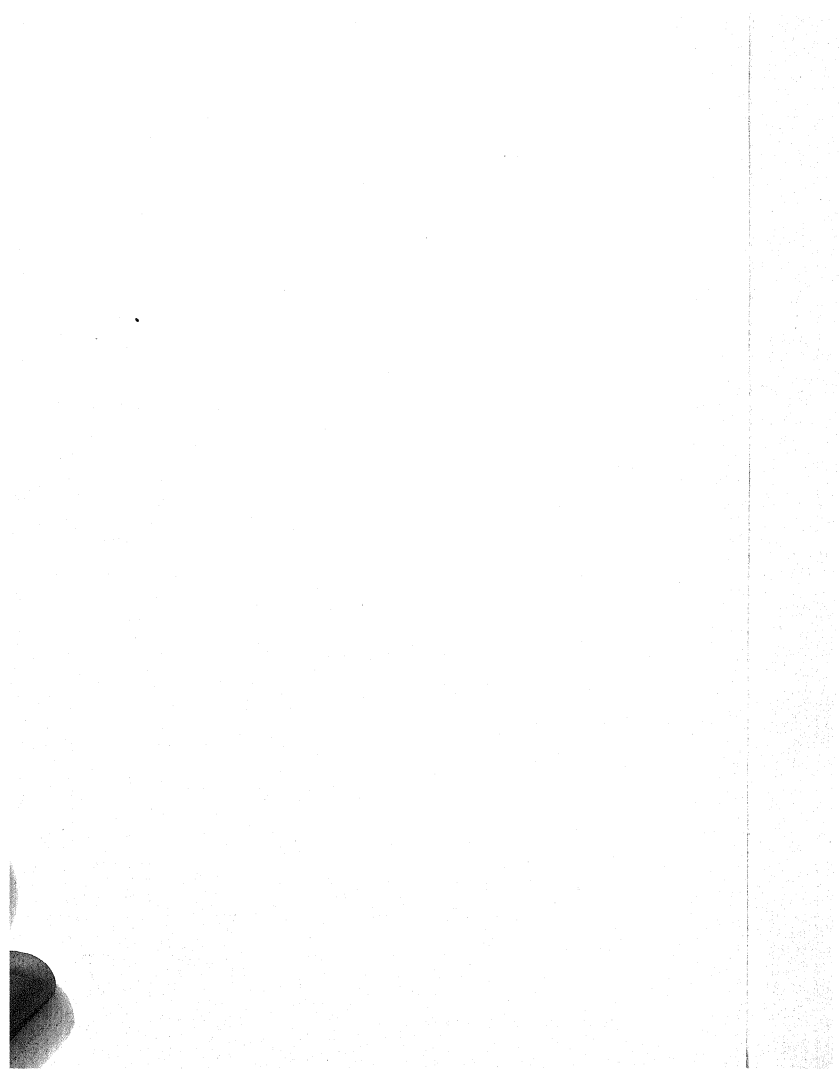
FIG. 43.—Egg with male nucleus not yet in contact with female nucleus; centrosomes in preceding and following sections above and below egg nucleus; $\times 1000$.

FIG. 44.—Male and female nuclei in contact; no centrosomes apparent; $\times 1000$.

FIG. 45.—Male and female nuclei in contact; centrosomes visible at opposite poles of egg nucleus; $\times 1000$.







CYTOLOGICAL STUDY OF MEIOSIS IN ANTHERS OF *OENOTHERA MURICATA*

RALPH E. CLELAND

(WITH PLATES III, IV)

Introduction

The species to be discussed in this paper is the *O. muricata* of DE VRIES' *Mutationstheorie* and *Gruppenweise Artbildung*, a species that BARTLETT (1) considers to be different from *O. muricata* Linn., and to which he has given the name *O. syrticola*. For certain reasons I shall retain the name *muricata*, but wish to make it clear that we shall be dealing with the *O. muricata* of DE VRIES' cultures.

Material was collected during the summers of 1919 and 1920 in the experimental gardens of B. M. DAVIS. A number of collections were made from several individuals. The strain chosen was a selfed line that originated in 1912 from seed obtained from DE VRIES. The most satisfactory fixative was found to be one of ALLEN's modifications of Bouin's solution (CLELAND 4). Unmodified Bouin gave good results also, but strong and weak Flemming both failed. The critical stages of late prophase and the heterotypic metaphase were plentifully obtained in three of the collections. Two of these were made in 1919, and include material from several individuals. The third, made in 1920, was obtained wholly from one plant.

Description

The earlier stages in meiosis have proved to be essentially identical in the various species of *Oenothera* that I have thus far described in detail. Nevertheless I have thought it advisable to examine them with care in *muricata* also. This study, however, has led to no new discoveries. So far as I can learn, there is nothing in the appearance or behavior of the pollen mother cells during the stages up to and including second contraction to distinguish *muricata* from the rest of the species investigated; consequently it will be unnecessary at this time to give a detailed account of early prophase.

Earlier papers may be referred to for figures and descriptions, which will apply to *muricata* as well. I wish merely to emphasize the fact that all of these stages give evidence that the meiotic process is telosynaptic rather than parasynaptic. In the presynizetic period, as well as in synizesis, the amount of parallelism that may be seen among the threads is not greater than would naturally be expected as a result of the contractions and condensation going on at this time. In the hollow spireme stage the threads are uniformly single (fig. 1); a case of parallelism is a rarity. The frequent appearance of splits or of approximations such as characterize the diplotene stage in many organisms is conspicuous by its absence. Furthermore, second contraction seems to witness to the presence of telosynapsis, inasmuch as splits in the threads fail to appear during this stage, and the chromosomes seem to be formed by a segmentation of the spireme as a whole, rather than from split halves of the threads. In early contraction stages, there are a number of radiating peripheral loops (fig. 2), which, as condensation proceeds, are more and more reduced, in many cases becoming at last entirely absorbed into the contracted mass at the center. In many cases the loops do not shrink to the same degree, however, so that they are visible throughout the whole of the contraction period (figs. 3-5), and it is possible to trace the direct transformation of certain of the univalent chromosomes from the exposed portions of these. The threads composing these loops, therefore, are without much doubt univalent in character.

LATE PROPHASE.—With the unfolding of the second contraction knot, there appear for the first time peculiarities in the behavior of the chromatin material which distinguish *muricata* from the other species of *Oenothera* so far studied cytologically. It soon becomes evident that the univalent chromosomes which are emerging, for the most part at least, are unpaired and attached end to end like a string of sausages. In a short time the chromosomes begin to take the form of a figure 8, four chromosomes in one circle and ten in the other, the circles being attached at one point. This attachment breaks shortly, however, and the final arrangement is found to be a single closed circle of fourteen chromosomes (figs. 6-10). In some cases the circle breaks open, and a chain of fourteen chromosomes instead of a circle is the result (figs. 11, 12). There are no paired

chromosomes at this stage. In this respect, *muricata* resembles *biennis* and *biennis sulfurea* (CLELAND 6), the last two, however, possessing two circles instead of one. This stage corresponds to what is called diakinesis in most plants, but the entire absence of paired homologous chromosomes is in complete contrast with the close pairing and wide separation of the pairs so characteristically found in other forms. The term diakinesis can hardly be used in connection with *O. muricata*.

It is obvious that the larger the circle of chromosomes, the more difficult it will be to find cells in which the circle is both intact and wholly clear. In the first place, such an appearance may only be expected in cells whose nuclei are uncut and complete in one section. In the second place, a circle of this size is naturally much contorted as it lies within the nucleus, and, except in unusually favorable cases, certain parts of the spireme will lie above other parts, and make it difficult or impossible to trace out the whole circle chromosome by chromosome. Consequently it is not to be expected that the whole of the intact circle will be observed in a large percentage of the cells examined. There should be a considerable proportion of cells, however, in which the end to end arrangement can be observed in the case of a large majority of the chromosomes; and almost every cell should display at least some of the chromosomes thus attached. Moreover, in no case should a single instance of paired chromosomes be found. A tabulation of the best cells discovered is presented in table I. Only those concerning which there was no question of interpretation have been included in this table. The positions on the slides have been recorded in the case of most of them, so that they can be found again.

In addition to the cells included in this table, hundreds of others have been studied in which smaller parts of the circle or chain have been visible. Five or six chromosomes can often be seen in one part of a nucleus attached end to end, and in another region three or four more are found similarly joined; but a tangle in the chain at one point makes it impossible to complete the picture. Such cells have not been included in table I. On the negative side, it should be emphasized that the most careful search has failed to reveal a single instance of paired chromosomes. Furthermore, there has been no

evidence of the existence of more than one circle or chain in any of the cells studied. On the other hand, we have a certain number of cells in which all fourteen chromosomes are clearly seen, and in all of these the whole chromosome complex is arranged into a single circle or chain. In addition, most of the other cells furnish evidence, more or less complete, of the entire absence of pairing, and the constant arrangement of the chromosomes into one series. I think that it may safely be assumed, therefore, that the chromosome arrangement at

TABLE I

CHROMOSOME ARRANGEMENT IN LATE PROPHASE IN *OENOTHERA MURICATA*

DESCRIPTION	NUMBER OF CELLS
Circle of 14, intact.....	21
Chain of 14.....	11
	<hr/>
Total..	32
13 chromosomes in continuous end to end series, fourteenth missing..	3
12 chromosomes in continuous end to end series, others missing.....	11
11 chromosomes in continuous end to end series, others missing.....	7
10 chromosomes in continuous end to end series, others missing.....	15
9 chromosomes in continuous end to end series, others missing.....	12
8 chromosomes in continuous end to end series, others missing.....	13
7 chromosomes in continuous end to end series, others missing.....	10
	<hr/>
Total..	71
	<hr/>
	103

this time is constant, and typical of the species; and, in this respect, *muricata* resembles the species previously studied.

The stage under discussion lasts a relatively short time. It marks the final disappearance of the nucleolus, and in addition is characterized by a gradual reduction in size of the chromosomes. For a time they may vary slightly in size and form. These differences gradually disappear, however, as the chromosomes become reduced, until finally they are uniform and individually indistinguishable. I am strongly inclined to believe that no significance is to be attached to form and size differences observed during this period. Such slight variations as are present are bound to occur as the chromosomes become smaller, unless the rate of shrinkage is precisely the same in the various chromosomes, which is extremely unlikely.

HETEROTYPIC METAPHASE.—A multipolar spindle is formed at the end of prophase, closely investing the nucleus, of which the membrane finally disappears. The spindle fibers penetrate the substance of the erstwhile nucleus, and some become attached to the chromosomes. These are now greatly reduced in size, but are still attached end to end, and the circle is still intact. So long as the spindle is multipolar in form, the circle lies in a heap in its center, without visible orientation (fig. 13). As it becomes bipolar, however, the chromosomes comprising the circle begin to assume definite positions with reference to the poles. Spindle fibers are found to have attached themselves in such a way that adjacent chromosomes are destined to pass to opposite poles, every second chromosome, therefore, being caught by fibers leading to the same pole (figs. 14-17). The behavior is identical with that described already for *O. franciscana sulfurea* (CLELAND 2), and for *O. biennis* and *O. biennis sulfurea* (6). In metaphase the same striking regularity is found that is so characteristic of these species. The chromosomes, attached to the fibers at about the midregion, are V-shaped. The fourteen V's are attached end to end, with the apices of adjacent chromosomes pointing to opposite poles. A regular zigzag appearance is thus observed, the circle as a whole lying horizontally across the equatorial region.

Owing to the fact that all fourteen chromosomes are associated into one group, making a rather extensive circle or chain, some contortion (as seen in polar view) is usually present. The commonest appearance presented in polar view is that of a circle, one side of which has been pushed in toward the center. Such an arrangement makes it more difficult to get complete pictures in side view, inasmuch as it may result in as many as four chromosomes lying one over the other at a certain point. It is often difficult, if not impossible, to distinguish between these with certainty, and it is still harder in such cases to distinguish the attachments between adjacent chromosomes. For this reason, the percentage of cells in which it is possible to trace out the whole circle is somewhat smaller than it would be if there were no such contortion.

In *muricata*, as in other species with large circles, a certain proportion of cases is found with irregularities in this zigzag arrangement. These cases conform to the types described in former papers.

Two adjacent chromosomes at one point in the circle may pass to the same rather than to opposite poles, and at another point two other adjacent chromosomes are found to pass to the other pole (fig. 18). Or a chromosome may be suspended between the poles, without any fiber attachment, its next neighbors on either side passing to opposite poles, and an adjacent pair at some other point passing to one pole, rather than separating. Or again, two chromosomes in different parts of the circle may be suspended (fig. 19). It is perhaps not possible to state accurately the percentage of cases in which irregularity occurs, inasmuch as the circles in a large proportion of the cells are incapable of certain and complete resolution. Some idea may be obtained indirectly, however, through a study of interkinesis, and a determination of the percentage of cells showing irregular chromosome numbers. Out of 683 such cells, sixty-two were found in which the number of chromosomes in one nucleus was six, and in the other eight, or a little less than 10 per cent. Inasmuch as irregularities in the zigzag arrangement do not necessarily result in unequal numerical distribution, however, it is to be expected that the total of irregularities will be somewhat higher than 10 per cent. This is borne out by the data in table II.

In order to make the evidence upon which I have based the description of the heterotypic metaphase as quantitative as possible, a complete survey has been made of the best slides containing this stage. Every cell in metaphase has been examined, and the condition noted in all cases where the cells were complete and in side view, and sufficiently clear to afford evidence of the chromosome arrangement. The result is contained in table II, which gives some idea of the probable percentage with which irregularity occurs in the zigzag arrangement. In part A of the table, the percentage of cells containing irregularity is 24.1. In parts B and C, the percentage of cells showing eight or more visible chromosomes in one continuous series is 22.2. Cells in which the visible chromosomes were located in different parts of the circle, down to and including those showing four in one region and four in another, display irregularity in 20 per cent of the cases. As a whole, therefore, the data suggest that irregularity in the zigzag arrangement occurs in approximately one-fifth of the cells.

Table II also presents in condensed form the exact data upon which are based my statements to the effect that the persistence of the circle and the presence of the zigzag arrangement are normal features of the heterotypic metaphase in this species. These state-

TABLE II

SURVEY OF CELLS IN HETEROTYPIC METAPHASE IN *OENOTHERA MURICATA*

NUMBER OF CHROMOSOMES CLEARLY ATTACHED END TO END, AND NATURE OF ATTACHMENT	NUMBER OF CELLS
A. All chromosomes visible	
Circle of 14, complete and regularly zigzag	34
Chain of 14, complete and regularly zigzag	10
	—
Total . .	44
Circle of 14, complete, with irregularity in arrangement	8
Chain of 14, complete, with irregularity in arrangement	6
	—
Total . .	14
	58
B. Some of circle obscure, but all visible chromosomes showing regular zigzag arrangement	
All visible chromosomes in one series	
Chromosomes	
13	1
12	17
11	11
10	20
9	24
8	18
7	19
6	26
5	14
Visible chromosomes in more than one series, separated by obscure chromosomes	
Chromosomes	
8 and 4	2
8 and 3	1
7 and 3	2
6 and 5	3
6 and 4	4
6 and 3	3
5 and 5	2
5 and 4	4
5 and 3	4
4 and 4	3
4 and 3	6
4, 4, and 4	1
	—
Total . .	185

TABLE II—*Continued*

NUMBER OF CHROMOSOMES CLEARLY ATTACHED END TO END, AND NATURE OF ATTACHMENT	NUMBER OF CELLS
C. Some of circle obscure, but irregularity visible in zigzag arrangement	
Visible chromosomes	
13	1
12	6
11	2
10	8
9	5
8	4
7	6
6	4
5	3
7 and 4	1
6 and 3	1
5 and 4	1
5 and 3	2
4 and 4	2
Total..	46

ments seem to be justified in view of the fact that in practically one-fifth of the cells it has been possible to trace out the entire circle or chain, and in most of the other cells a majority of the chromosomes has been visible and their arrangement in metaphase in conformity with the rule suggested.

The circle remains intact until anaphase, at which time adjacent chromosomes are pulled to opposite poles. The V-shaped chromosomes keep together as they pass to the poles, so that one practically never sees a lagging one. Very rarely, some evidence of a longitudinal split may be observed as the chromosomes near the poles. As a rule, however, this does not appear until later. The daughter nuclei are constituted after the manner typical of *Oenothera*. When the normal number of chromosomes is involved, one tends to occupy the center, with the others around the periphery of the nucleus (fig. 20). For a time they may be slightly attached, but after the nuclear membrane has been formed, the attachment is quickly lost because a period of growth ensues, the nucleus becoming much larger and the chromosomes consequently more widely separated. The chromosomes also expand at the same time, although to a lesser degree than the nucleus as a whole. The ends of the chromosomes elon-

gate, and it is at this point that it is generally seen for the first time that they have undergone a longitudinal split (fig. 21). As elongation proceeds, the ends of the split halves diverge, so that the whole chromosome comes shortly to resemble a Maltese cross. In mid-interkinesis the time of maximum expansion is reached (figs. 22-24). The chromosomes are in general X-shaped. They have swollen tips and centers, and sometimes there seems to be another swollen portion midway between end and center, although this cannot always be seen.

One or more spherical nucleoli make their appearance in early interkinesis (figs. 22, 23). They are in contact with the chromosomes, remain small and colorless, and disappear before the nuclei disorganize. Their relation to the chromosomes strongly suggests that they originate from these, and hence arise *de novo*.

Very little need be said about the remaining stages, which differ in no way from corresponding stages in the other species of *Oenothera* examined. Chromosomes and nuclei pass through a period of contraction, the X-shape being retained until after the disappearance of the membranes. Multipolar spindles give place to bipolar ones, and the chromosomes are gathered in a regular manner to the plates. The two figures are constituted simultaneously, the angle which they bear to each other varying (fig. 25). As seen from the side in metaphase, each chromosome is distinctly a double affair, and in turn the halves are often constricted to some extent at the center. Anaphases are normal, and the granddaughter nuclei are formed as were the daughter nuclei. At first the chromosomes are distinct and separate (fig. 26), but a process of elongation occurs as the nuclei increase in size and delicate threads are sent out from the various chromosomes, uniting to form a loose and delicate reticulum (figs. 27, 28). A passage of stainable material from the chromosomes into these threads finally results in an irregular network, and the loss of visible individuality on the part of the chromosomes.

The four spores are formed simultaneously by a process of invagination of the cytoplasm from without inward, along the lines of the future cleavage. These do not seem to penetrate to the center, however, but delicate cleavage planes appear, completing the separation. This is particularly interesting in view of the absence of cell

plates and the early disappearance of interzonal fibers after the homotypic divisions.

Discussion

O. muricata is probably the most striking species yet examined, so far as its cytology is concerned. It shares with *O. biennis* and *O. biennis sulfurea* the distinction of having no pairing whatever of homologues in the late heterotypic prophase; but in the fact that all fourteen chromosomes are found in the same circle, this species represents the extreme limit to which the development of circles can attain. One cannot help feeling that a chromosome behavior so unusual must be full of significance, especially as *Oenothera* is unique from the genetical standpoint also. I have discussed previously in some detail what seems to me to be the probable significance of the phenomena observed (6). A brief review of some of the principal points, however, may not be out of the way in connection with this study of *muricata*.

In the first place, it is quite certain that the chromosomes are not arranged hit or miss within the circle. They must be so placed that the method of distribution to the poles in the heterotypic anaphase will ordinarily result in the separation of the members of each pair of homologues; otherwise non-disjunction would be the result in an impossibly large number of cases. It is possible to calculate what the chances of a distribution free from non-disjunction will be in a circle of fourteen, provided the chromosomes are placed entirely according to chance, remembering that the members of a pair of homologues will be separated in the reduction division (with every second chromosome going to the same pole) only when they are adjacent in the circle, or separated from each other by intervals of two, four, or six places. The total number of ways in which the fourteen chromosomes, acting as units, can be arranged within a circle of fourteen is 13, and the number of arrangements that will result in the separation of the members of every pair of chromosomes, and complete freedom from non-disjunction, will be 7. 6. The ratio of these is 1716:1. Only one cell out of 1717, on the average, will be entirely free from non-disjunction if the chromosomes are arranged merely by chance within the circle. There is,

however, no evidence of any such enormous prevalence of non-disjunction in *O. muricata* or any other species of *Oenothera*. We are forced, therefore, to abandon the idea that the position of the chromosomes in the circle is a mere matter of chance. On the other hand, if it be true that each pair of homologous chromosomes is definitely placed within the circle, then it is most likely that the same applies to each univalent chromosome also, and that each has its own place in relation to the other chromosomes; and the circle, which seems to be uniformly present throughout the species, is uniformly constructed as well.

Coupled with this is the clearly observed fact of the zigzag arrangement in the heterotypic metaphase, and the regular passage of every second chromosome to the same pole. If the chromosomes are arranged according to a set scheme within the circle in all cells, then such a distribution can have but one result. It is obvious that in every cell in which the zigzag arrangement is found to be wholly regular, the same chromosomes will proceed together to the same pole. Those passing to one pole are therefore as much linked, so far as the genetical effect produced is concerned, as though they were all a part of one chromosome. Hence we may say that the circle as a whole acts like one large chromosome pair, the members of which, each consisting of seven chromosomes, are separated to opposite poles. If a large amount of heterozygosity exists within the circle, as I believe is the case, the complexes resulting from the heterotypic division will differ in certain respects. As there are but two kinds of complex, there can be only two possible sorts of gamete. Owing probably to the presence of a balanced lethal situation, only the combination of the two complexes can survive, and so the species will breed true in the main. This seems to be the most obvious explanation of the relation between the undoubtedly unique chromosome behavior and the equally peculiar genetical phenomena, not alone in *muricata*, but also in various of the other species of *Oenothera*.

Irregularities in the zigzag arrangement, if the situation is as described, will alter the normal genetical result wherever they occur. In some cases they will lead to an abnormal numerical distribution, eight chromosomes passing into one cell and six into the

other. Sperms containing eight chromosomes may be capable of functioning and giving rise to fifteen chromosome individuals, although I am not acquainted with any evidence for the existence of fifteen chromosome plants in selfed lines of *muricata*. As for those possessing but six chromosomes, it is probable, judging from the entire lack of reported thirteen chromosome evening primroses, that in all cases they will be unable to function.

Irregularities in metaphase, however, do not necessarily lead to disturbance in the number of chromosomes distributed. In many cases the number passing to the poles will be normal, but both members of one of the pairs of homologous chromosomes will pass to the same pole, with the consequent absence of either member of some other pair in the resultant cell (6). Such cells, lacking entirely a representative of one of the pairs, will probably fail to develop functional sperms.

Other cells, as I have brought out in detail in connection with *O. biennis* and *O. biennis sulfurea*, will not only have the normal number of chromosomes, but a representative of every pair of homologues as well; but because of the irregularity in the zigzag arrangement, one or more chromosomes normally belonging to one complex will be found to have exchanged places with their homologues, and so be situated in the other complex. I have previously referred to this under the name of "interchromosomal crossing over." The resultant complexes, possessing a complete set of chromosomes, in most cases at least should prove functional, but the genetical result of this exchange ought theoretically to be of considerable importance.

In crosses, plants resulting from the union of sperms containing such complexes with normal eggs should develop into cross-overs. In selfed lines new characters should appear, and others that are normally present should fail to appear, for individuals arising from the union of such irregular complexes with the normal type would as a result have one or more pairs of identical chromosomes, depending upon the number exchanged. To illustrate, if we represent one complex by the letters ABCDEFG and the other as A'B'C'D'E'F'G'; and if, as a result of irregular distribution, A and A' are exchanged in a certain cell, a plant arising from the union of a sperm having the

complex A'BCDEFG with the normal A'-G' would have two A' chromosomes, but no A. It being a selfed line, the two A' chromosomes would probably be identical. Dominant characters depending upon the presence of genes in A would be lacking, and recessive characters depending upon genes in A', and which had never before been visible because of the enforced heterozygosity of the species, could now express themselves. In cases where the exchange affects a single chromosome pair only, the difference between the resultant individual and those arising normally might be very slight. Where the number involved is greater, however, the effect should be more striking.

I wish at this time to emphasize the tentative nature of these suggestions. There is no question in regard to the cytological facts. They are clearly observed and easily demonstrable, but it is possible that the attempt to apply these phenomena to the genetical situation has not been entirely in keeping with the facts as they will in time be revealed. In the light of present knowledge, however, they seem to point the way toward a solution, based upon chromosome behavior, of many of the problems presented by the genetical behavior of *Oenothera*. It is hoped that they will at least serve to stimulate research in this direction, and that cytological investigation will develop many concrete data upon which to base further research. It is hardly necessary to suggest the desirability of *Oenothera* geneticists knowing so far as possible the chromosome arrangement and behavior in meiosis in the case of every plant used in breeding work.

Summary

1. Early stages in the heterotypic prophase in every way resemble those found in previously described species. They give evidence of the presence of telosynapsis as opposed to parasynapsis.
2. The stage known in most plants as diakinesis is absent in this species. There is no pairing of homologous chromosomes, but instead all of the fourteen univalent chromosomes are found to be united end to end to form a large closed circle, or occasionally an open chain. This configuration appears to be normally present and typical of the species.
3. The circle remains intact throughout the heterotypic meta-

phase, and spindle fiber attachments are made in such a way that adjacent chromosomes are pulled toward opposite poles. In general, the position of the circle in the spindle is horizontal, but since the chromosomes are alternately pulled to upper and lower poles, it takes on a regular zigzag appearance as seen from the side.

4. Irregularities occur in the zigzag arrangement in approximately 20 per cent of the cells.

5. The circle breaks up with the pulling apart of the chromosomes in anaphase. Subsequent stages in every respect are like those previously described for other species.

6. The chromosomes are without much doubt arranged within the circle according to a fixed scheme, for otherwise a certain amount of non-disjunction would occur almost every time that a reduction division took place.

7. If it be true that the chromosomes are arranged in a definite fashion within the circle in all cells, then it naturally follows that as a result of the normal separation of adjacent chromosomes in heterotypic anaphase the same chromosome complexes will in every case be formed at the poles.

8. There will then be but two kinds of complex formed, and two kinds of gamete; and the presence of balanced lethals probably accounts for the fact that neither can function in the homozygous condition, but only a combination of the two can be successful.

9. Irregularities in the zigzag arrangement doubtless lead to abnormal distribution of the chromosomes, which may result in functionless spores or gametes when the resultant complexes lack a representative of one or more of the chromosome pairs; but in many cases irregularities merely cause an exchange of the members of one or more homologous pairs from one complex to the other, and the complexes thus formed being complete, the cells containing them may be capable of functioning.

10. Such complexes, although complete, are abnormal. If they function in crosses with other species, they may result in the appearance of cross-over individuals. In selfed lines the union of such complexes with normal ones containing the proper lethal may result in a plant deviating more or less from the usual type.

In conclusion, I wish to thank Professor B. M. DAVIS for allow-

ing me to collect material from his cultures, and for many expressions of interest in the work. Grateful acknowledgment is also made to the officers of the Marine Biological Laboratory, Woods Hole, Massachusetts, especially to Professor I. F. LEWIS, for facilities placed at my disposal during several successive summers.

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EXPLANATION OF PLATES III, IV

Figures were drawn with the aid of a camera lucida, using a Spencer compound binocular microscope, and a Bausch and Lomb 1-12 apochromatic objective with 25X compensating oculars. They have been reduced one-fourth in reproduction. Present magnification approximately 2800 diameters.

PLATE III

FIG. 1.—Hollow spireme; threads unsplit.

FIG. 2.—Early second contraction, showing growing condensation in middle of nucleus and peripheral loops.

FIGS. 3-5.—Late second contraction stages.

FIGS. 6-10.—Circle of fourteen chromosomes in late prophase, corresponding to period generally called diakinesis.

FIGS. 11, 12.—Circle broken open and resulting in chain of fourteen chromosomes; late prophase.

PLATE IV

FIG. 13.—Multipolar spindle; circle of fourteen chromosomes still intact.

FIGS. 14-16.—Heterotypic metaphase with circle of fourteen chromosomes; adjacent chromosomes ready to pass to opposite poles, giving zigzag appearance (spindle omitted in figs. 14 and 15).

FIG. 17.—Chain of fourteen chromosomes in heterotypic metaphase, regularly zigzag (spindle omitted).

FIG. 18.—Irregularity in zigzag arrangement in heterotypic metaphase (spindle omitted); two adjacent chromosomes at left end ready to proceed together to upper pole, and another pair at right end about to pass to lower pole.

FIG. 19.—Another irregularity in a chain of fourteen chromosomes; two at different points suspended in center of spindle without visible fiber attachment.

FIG. 20.—Early telophase; chromosomes gathered at pole and attached, but nuclear membrane as yet unformed.

FIG. 21.—Early interkinesis; chromosomes beginning to take X-form.

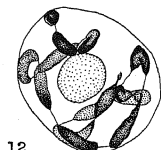
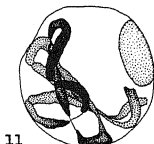
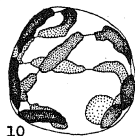
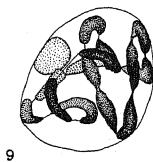
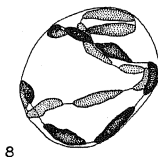
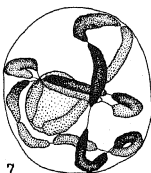
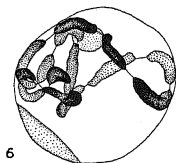
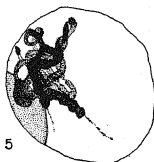
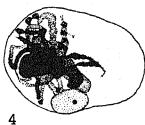
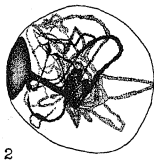
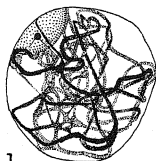
FIGS. 22, 23.—Mid-interkinesis.

FIG. 24.—Mid-interkinesis; both nuclei in one pollen mother cell; six chromosomes in one nucleus and eight in other.

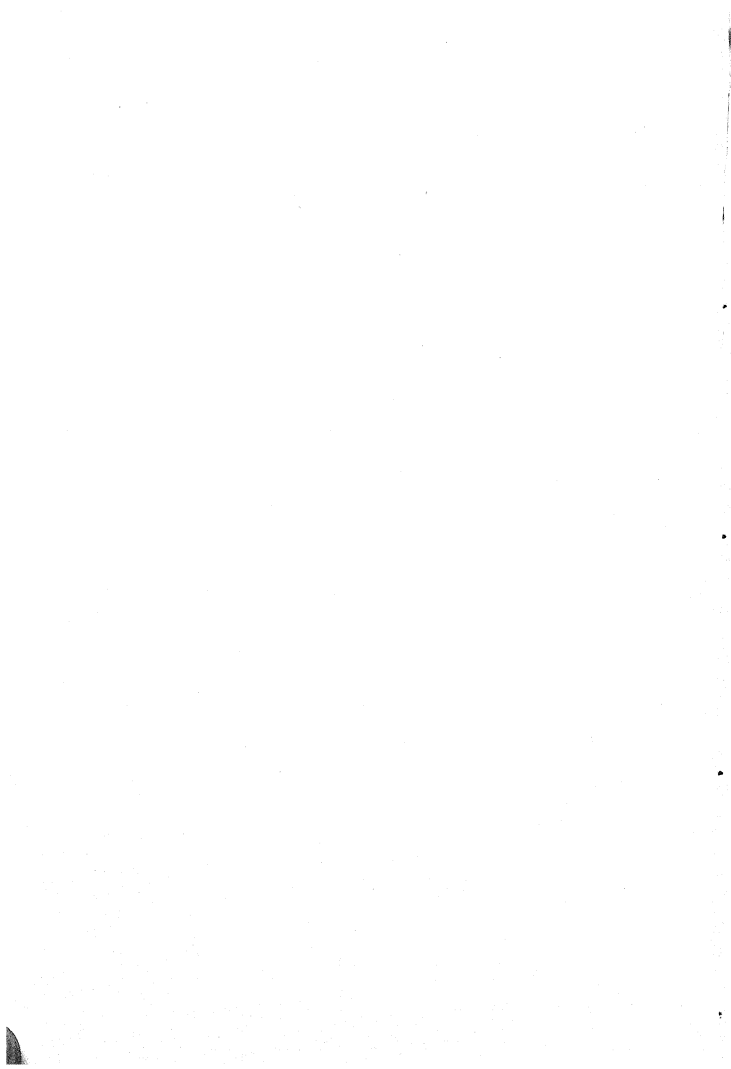
FIG. 25.—Homotypic metaphase, side view of one plate, polar view of other.

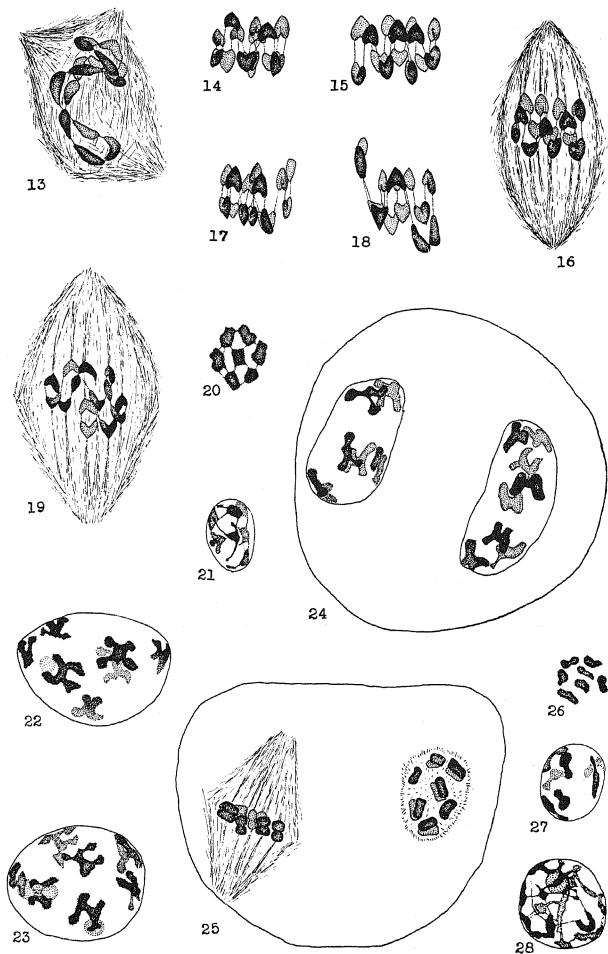
FIG. 26.—Second telophase.

FIGS. 27, 28.—Passage of granddaughter nucleus into resting condition; individuality of chromosomes lost sight of in fig. 28.



CLELAND on OENOTHERA MURICATA







ORGANIZATION AND SIGNIFICANCE OF LENTICELS IN DICOTYLEDONS

I. LENTICELS IN RELATION TO AGGREGATE AND COMPOUND STORAGE RAYS IN WOODY STEMS. LENTICELS AND ROOTS.¹

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(WITH PLATES V, VI)

Introduction

In a recent joint article by Professor JEFFREY and the writer,² on the morphological and evolutionary significance of the parichnos, it was pointed out that this structure was probably widespread as an organ of aeration in ancient arboreal forms. This belief is supported by the general existence of a parichnos in the Lepidodendraceae accompanying each foliar trace into the leaf, and there becoming confluent with the intercellular spaces of the mesophyll. A reason for the presence of such an aerating channel was found in the absence of cauline stomata. Also the retention of the paired parichnos scars was explained as permanent aerating structures after the fall of the leaves. These paired structures have indeed been considered as primitive lenticels (POTONÉ 10, 11; SCOTT 14, 15). It was pointed out as further evidence of the general distribution of the parichnos in ancient forms, that there are devices initially present in certain conifers and in *Ginkgo biloba*, as well as in certain species of *Equisetum* (JEFFREY 5), which were clearly parichnos-like in nature. In the conifers and *Ginkgo* the exact conditions of the Lepidodendraceae are duplicated, both with regard to the actual form and position of the parichnos, and the general absence of cauline stomata.³ In these forms the parichnos is but transitory, soon being lost because of the increased diameter of the stele, but to supporters of the bio-

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

² Unpublished. Ann. Botany.

³ Culine stomata were found only on the decurrent leaf bases of *Taxus canadensis* and *T. cuspidata*.

genetic law its initial presence indicates the existence of a parichnos as a permanent structure in the ancestors of the conifers and *Ginkgo*, as well as of the Equisetaceae. Unfortunately, there is at present inadequate fossil material to prove this fact conclusively. It is significant also that the primary lenticels on the stems of the conifers invariably appear with the fall of the leaves, as paired structures on either side of the foliar bundle scar, just as in the Lepidodendraceae. This fact is true even in those conifers when the parichnos is no longer present. Moreover, such a condition is the permanent one in coniferous roots.

Since the parichnos is present in only a few genera of conifers, and in these early becomes an obsolete structure, aeration must be furthered by other means. The primitive lenticels, therefore, early acquire secondary relations and connections, and in this readjustment they become transversely elongated, thus enabling them to aerate the maximum number of vertically short rays and thence the other parenchymatous tissue of the stele. Furthermore, it is of interest that other lenticels of haphazard distribution later appear on coniferous stems, thus providing air for the ever enlarging stele with its continually increasing number of wood rays. With these ideas in mind, attention is directed to the angiosperms.

Materials and methods

The results of this research are based on a study of some 279 species and 35 varieties of dicotyledons, representing 89 genera of 35 families, and comprising both exotic and indigenous forms. Data were assembled from all these forms, entailing a study of the lenticular organs of both stem and root. Not only was information gathered on the size, shape, type, and distribution of these organs, but material was also collected for laboratory investigation.

With the material of both stems and roots, pieces of various ages were obtained. These specimens were blocked up in small segments, averaging 1 cm. long, and preserved in 70 per cent alcohol. When used, the material was halved, one portion being softened, imbedded, sectioned, and stained after the generally accepted methods employed for ligneous structures (JEFFREY 6). The second portion was treated for removal of epidermis and periderm. This meth-

od is an unpublished one, devised by JEFFREY for this purpose, and is especially applicable in investigating the presence or absence of stomata. It consists essentially of subjecting the material to the action of a diluted saturated solution of sodium chlorate in hydrofluoric acid. The strength of the solution used is determined by the material to be macerated, and is ascertained by experience alone. It should not be used pure for this work, but according to demand. The stock solution is prepared by adding sodium chlorate crystals to concentrated hydrofluoric acid until there is a surplus of crystals present. A wax covered bottle is necessary as a container for this solution. When material is to be macerated, this also must be placed in wax covered bottles, which must be clean, and free from foreign liquids as well as solids. If not thoroughly devoid of extraneous matter, rapid oxidation ensues on addition of the maceration fluid, usually with the production of sufficient heat to melt the wax. This means that the glass is exposed to the vigorous attack of the acid and the material is thus frequently lost. After the material to be treated for removal of periderm or epidermis is placed in the bottles, a small amount of water is added. The maceration fluid is then poured on to a desired strength, varying from about 20 to 60 per cent, according to the age of the twigs and the nature of the periderm. Young material for epidermal removal requires a weaker solution, whereas older material with a heavy periderm necessitates a more concentrated reagent.

After a few days in this fluid, the time being a variable factor, the material is washed thoroughly under running water. It is then found that the cork cambial or subepidermal cells have been macerated to a degree permitting the removal of the periderm or epidermis. If these sections are then shaken vigorously in water, to remove adhering bits of cortex or phelloderm, they are ready for bleaching. This procedure is a replica of the last, the same reagent being used as for maceration, only in weaker solution, 10-20 per cent being of adequate strength. Careful watching of the material for a day or two shows that it has become absolutely white. After again thoroughly ridding the material of all bleaching fluid under running water, it is ready for staining. As a result of several trials, the iron-alum haematoxylin of HAIDENHAIN proved the most satisfactory

stain, when used in small quantities over long intervals of time. A counter stain, however, was found to be of little additional value. After staining, the sections, whether epidermal or peridermal, were then dehydrated, cleared in benzol, and mounted in balsam according to the usual methods.

This procedure was used for removal and study of the epidermis and the periderm of many different species of conifers and angiosperms. It made possible the decision as to whether stomata were present or absent, whether lenticels first appeared over these or not, and the nature of the lenticels themselves.

Angiosperms

In the angiosperms anatomical conditions are very different from those found in even the highest conifers. In no case are there indications of such an ancient structure as the parichnos, even in the most conservative organs. Such a structure is not a requisite to survival because of the general existence of stomata on the stems of angiosperms.⁴ Because of these organs, the epidermis is viable to gases, and aeration is thus made possible until the formation of periderm is initiated. Very probably the parichnos and generally distributed cauline stomata have never been correlated structures at any time in plant evolution. STAHL (16) differentiated lenticels as found in angiosperms into those of primary or secondary nature. The former, he claimed, originated under stomata, as previously stated by UNGER (19) (although he later seemingly discarded this belief) and TRÉCUL (18). Later workers all agree on this point.

Since the cauline lenticular structures of the angiosperms are primarily substomatal in origin, their distribution is haphazard and at first entirely without internal correlations. Their appearance, as STAHL showed, is usually followed promptly by the origin of the initial peridermic sheath. He pointed out, however, that in cases where an abnormally heavy cuticle exists, the primary periderm

⁴ DEVAUX (3) mentions eight species, widely separated in affinity, in which he failed to discover stomata. In no instance has the writer found them absent in the material collected for this research, although none of the forms named by DEVAUX was contained therein. It is worthy of note that the same investigator found cauline stomata invariably much larger, although fewer in number, than those on the leaves of the same species.

may never develop at all. The writer found several instances of this condition.

This primary periderm may be one of lasting nature, and even a permanent structure, although most frequently it is quickly replaced as a result of secondary phellogenic activities within. If the initial sheath is persistent, the lenticels of stomatal origin will also become lasting structures. It is common, however, for them to lose their initial limitation to the region of the substomatal cavity, and to become greatly increased in size, with other relationships than earlier shown. Thus primary structures may remain as such, or they may assume secondary proportions and connections. *Betula* is typical of this interesting situation. STAHL (16) asserted that he could not find a single cauline stoma in strips of young birch bark that had not given rise to a lenticel. It is worthy of note that, despite their substomatal origin and their persistence as primary structures, they early cease to be limited by stomatal confines and become extended transversely, so that on older trees they appear frequently as horizontal markings continuing around a large portion of the circumference.

The secondary relationships acquired by these lenticels are equally significant and exactly comparable with those possessed by the secondary structures which arise with the appearance of other periderm formations than the initial one. DEVAUX (3) stated that although primary lenticels showed definite relationships to stomata, those of secondary origin seemed devoid of any such connection. It is true that externally, as he viewed them, there is none apparent. Internally it will be shown that all lenticels of secondary relationship, whether of primary or secondary derivation, display the most intimate correlation with the storage rays, irrespective of the type and distribution of the latter.

The writer finds that secondarily modified, primary lenticels, and all secondary lenticels are capable of classification according to shape. There are those which are oriented transversely. These *transverse* lenticels, as the writer has labeled them, are more characteristic of the members of the lower angiospermous affinities, and the (generally conceded) less highly evolved genera of the higher families. Such exists in *Betula alba* var. *papyrifera* (fig. 1). The remain-

ing lenticel bearing forms, chiefly belonging to higher groups in the systematic scale, and the probable more highly evolved genera of the lower assemblages, possess what the writer calls *longitudinal* lenticels. These have the fissure elongated in the direction of axis. Exemplifying the latter is *Alnus rugosa* (fig. 4). At this point it will be well to mention that both of these types of lenticels have been referred to by different writers, but no significance was attached thereto. Many writers considered all mature lenticular structures extended crosswise, as is apparent from the works of DE CANDOLLE (2), MOHL (9), MEYEN (8), SCHACHT (13), SACHS (12), and others. Longitudinal orientations have seldom been noticed, although STAHL (16), WEISS (20), and DEVAUX (3) do mention them in specific instances. Certain of the earlier writers, as MOHL and MEYEN, did call attention to lenticels being longitudinal first and then transverse. These references undoubtedly were to primary lenticels, which early are always of this orientation, due to the elongation of the stomata from which they were derived. They may remain so or become transverse with maturity.

The classification of lenticels proposed by the present writer is not opposed to that of STAHL (16) nor to the modification suggested by DEVAUX (3). It is a result of another method of approach to the problem, as well as of the employment of very different criteria. That the two systems are not coincident will be apparent when it is mentioned that the writer finds both types of STAHL's lenticels in each of the transverse and longitudinal classes suggested herein. This will be evident as the various illustrations used in the work are referred to. The grouping as used in this research is intended to be suggestive of internal relationships which will be shown to exist between the lenticels and the storage rays within. The earlier types were based on the existence of lenticular structures as superficial organs, devoid of any internal connection. The illustrations in the sequel will make this point clear. Fig. 13 is a representation of a lenticel of *Alnus rugosa*, an example of STAHL's type I. The banded nature of the structure is clearly visible. A further example of this type is indicated in fig. 2, a lenticel of *Betula populifolia*. This differs from the last in remaining long closed, so far as ruptures in the clos-

ing layers are concerned. In *Alnus rugosa* only the inner three or four of these lamellations remain unbroken. In contrast to these two examples there are shown in fig. 14 two lenticels of the root of *Sambucus canadensis*. Although under low magnification, they can be seen to be of STAHL's type II, since the complementary tissue is clearly not lamellate, the cells appearing practically uniform in size.

That these earlier groupings were not as fundamental as supposed is suggested by KLEBAHN's (7) discovery of lenticels which did not conform to the limitations of either of STAHL's groups, but rather seemed between the two. Also, DEVAUX's experimental studies pointed to the same conclusion, when he found that by altering the humidity of the medium he could change the type of lenticel at will. These types are of some significance however. As KLEBAHN has pointed out, STAHL's first type is undoubtedly a more efficient protective organ than his second, and indicates a higher evolutionary status. The writer finds that the major part of the cauline lenticels studied in this research belong to this higher class, the second type (the diffuse lenticel) being the exception rather than the rule. In roots, only those of STAHL's second type have been found, possibly due to the conservative nature of this organ, or possibly, as asserted by DEVAUX, to the presence of moisture in the soil.

In discussing these modified primary and secondary cauline lenticels, since they show a close relationship to the rays within, they will be treated along with the form of storage ray present, whether aggregate, diffuse, or compound. Under the different types will be mentioned examples of both transverse and longitudinal types of lenticels, with an endeavor to point out internal anatomical reasons for the nature of the lenticel thereon occurring.

For the sake of convenience in designating the types of rays concerned, the terminology of JEFFREY (6) has been adopted. Storage structures composed of aggregations of uniseriate rays or multi-seriate rays of small dimension, in close proximity to each other, have been termed "aggregate." Such congeries of small rays extend for considerable distances vertically in the stem. Exemplifying this condition are many of the generally conceded lower angiosperms, for example, *Alnus incana* (fig. 10) and *A. japonica* (fig. 9). If, however, instead of the composite aggregation of small rays and fibers,

the storage structure appears as a homogeneous band of parenchymatous tissue, the ray is termed "compound." Such compound rays, rare in arboreal and fruticose forms, are found in at least the mature stems of *Quercus*, where they are greatly elongated vertically. Fig. 11 represents a transverse view of a small stem of *Quercus alba*, where a compound ray is shown. In general, however, the initial aggregations in woody angiosperms become diffused into multiseriate structures of varying width and height, more or less uniformly distributed throughout the stem. Such storage structures are spoken of as "diffuse" rays. The more primitive forms possessing diffuse rays tend to have the consequent multiseriate components vertically shorter than those in higher systematic groups.

In the present paper, cauline lenticels and their relationships will be discussed in species with aggregate or compound rays. A study of the lenticular situation in roots will also be reported. A later paper will complete the investigation with a summary of the situation in species possessing diffuse rays.

LENTICELS AND AGGREGATE STORAGE RAYS

The fact that there are definite internal conditions accompanying the presence of transverse lenticels, and that these conditions are materially different when longitudinal structures exist, can well be pointed out by reference to concrete examples.

In *Alnus* it is interesting that both types of lenticels exist in different species. Thus fig. 3 (left) illustrates the situation in *A. incana*. The lenticels here are clearly transverse as well as horizontally seriate. Fig. 3 (right) is a portion of a branch of *A. japonica*. The lenticels here are much as in the preceding species, although smaller and not horizontally seriate. In contrast to these two may be mentioned *A. rugosa*, two pieces of which are shown in fig. 4. Here is clearly indicated a longitudinal orientation in all the lenticular organs, although they exist in varying sizes. This difference in magnitude is a result of differential ages, such secondary lenticels increasing in number progressively with time.

It is instructive to compare internal conditions with these lenticular dispositions. Fig. 10 shows a transverse section of *Alnus incana*. Here four aggregate rays are clearly visible, and a fifth less

distinctly so. The lenticels, two in number, are also very apparent and close together. That an aggregate ray exists under each is noticeable, but it is equally noticeable that each lenticel not only subtends one of these, but many uniseriate structures as well. Such is the general situation in this species. Lenticels usually lie opposite one or more aggregate rays, but whatever co-ordination there exists between internal structures and lenticels is not a result of the influence of aggregate rays alone. An accompanying condition should be mentioned here. A short distance within the periderm the sclerotic pericycle is apparent, extending continuously across the field.

In *Alnus japonica* conditions are more or less the same, as shown in fig. 9. Here the single lenticel in the field is distinctly confronted by an aggregation of small rays; however, here again it is not confined in lateral extension to the limits of that ray, as it subtends several non-aggregated, uniseriate structures on either side as well. Here again also the sclerotic pericycle is continuous, being clearly visible between the outward ends of the rays and the periderm. In *A. rugosa*, as just mentioned, we find longitudinal lenticels. Here in cross-section (fig. 13) are portrayed two lenticels, each clearly subtending aggregations of rays. Moreover, in their vertical elongation these lenticels are much narrower, each more nearly corresponding to the width of an aggregate ray. It is also clear that the continuous pericycle of the other two species has now become thin and practically disrupted in places under the lenticels. Thus is assured a more direct communication between the lenticels and the rays, through the cortex.

To epitomize the situation as shown in the alders, it would seem that the primitive condition of relationship is found in *A. incana*. Here the lenticels are frequently in horizontal chains, related to uniseriate and aggregate rays alike. There seems to be no closer connection with the aggregations than with the more primitive structures, for the pericycle is uniformly heavy all the way around. In *A. japonica* another tendency becomes apparent. In spite of a great many series of sections having been cut, no case was found where a lenticel was not opposite an aggregate ray, although it is true that the lateral extension permitted it to confront those of uniseriate nature on either side as well. In this and the preceding spe-

cies, however, the pericycle remains continuous. Matters have progressed further in *A. rugosa*. Here the lenticels are clearly longitudinal, narrow in lateral extension, and unquestionably related to aggregate rays, and these alone. A few cases only were found where such lenticels were to any extent wider than the rays they subtend, but in every instance this larger lenticel was confronting two closely approximate aggregations. Moreover, the movement of gases in these regions between the lenticels and the rays is facilitated by the interruptions of the pericycle opposite the lenticels, although continuous elsewhere. Thus it seems that as the storage aggregation of rays has evolved, with its greater metabolic activities, there is an accompanying demand for an increased aeration of these structures to facilitate that accentuated metabolism.

These results are interesting also when the correlation is considered in relation to the extent of aggregation. *Alnus incana* is recognizably a primitive alder, with aggregations of uniseriate rays mostly, only occasional signs of their uniting to form multiseriations being visible (BAILEY 1, HOAR 4). On the other hand, HOAR shows that *A. japonica* has a higher aggregation, being largely composed of multiseriate units. *A. rugosa* is also of the latter type, with congeries of multiseriates for the greater part making up the storage ray. This correlation of internal ray structure, with the nature of the lenticels and distribution of the same, is most striking. In fig. 4 (right) is shown a section of a stem of *A. rugosa* in which rhytidome has appeared in the initial bark. At the base of the fissure, lenticels derived from the secondary periderm are apparent, clearly like their predecessors in orientation. It is significant that these are more or less in longitudinal rows. Investigation of such instances showed that they were lying along the aggregate ray which extends vertically for long distances in the stele. These vertical lines of lenticels are very apparent on older stems of *A. rugosa*, and are significant in this respect, since they still further increase the possibility of aeration of these storage organs.

Although *Alnus* alone has been treated thus far, similar conditions are found elsewhere. *Corylus*, also of the Betulaceae, is a very interesting genus. Fig. 8 shows a surface view of *C. americana*. Here the earlier secondary lenticels are clearly transverse. As the stem increases in diameter, however, many small longitudinal lenticels be-

come apparent, some even being found immediately on the already existing transverse lenticels. Although these are small, a careful examination of the figure will reveal their presence. Again internal studies appeared illuminating. The aggregate rays present are related to the transverse lenticels, as are those of *A. incana*, one lenticel often subtending several of these structures as well as a host of the uniseriate type. As these aggregations become more mature, and the components multiseriate, the longitudinal structures begin to appear. In no case was a lenticel of this type found otherwise than subtending an aggregate ray. Various species of *Corylus* showed this transitional condition. In *C. rostrata* the situation is the same, although the transverse lenticels are early replaced by longitudinal ones. *C. avellana* and *C. maxima* also duplicate these conditions.

Carpinus, of the same family, provides another case parallel to that in *Alnus*. *C. caroliniana* has broad, transverse lenticels, sometimes over aggregate rays, sometimes not. On the contrary, *C. japonica* has longitudinal lenticels distinctly opposite the aggregate rays, which are composed of multiseriate units.

These parallel instances serve to indicate that, with aggregate structures at least, there is a direct relation between the type of aerating device and the structure to be aerated. In general, the more highly evolved aggregate ray (that is, the one with multiseriate structures replacing those of uniseriate nature) has the more advanced type of lenticel, the longitudinal structure. With the localization of the lenticels in the region confronting the storage rays, the uniseriate structures lose their direct connection with the aerating devices. This is significant in the light of STRASBURGER'S (17) results. He found that in the majority of dicotyledonous trees and shrubs examined, these primitive rays were devoid of intercellular spaces. They were not starch bearing, and instead, like the erect cells of the multiseriate rays, were connected with the vessels by pits. He concluded that in these forms they served as adjusters of the sap flow in the plant, as did the erect cells already mentioned. This localizing of storage and aerating structures is accompanied usually by the thinning out of the pericycle, and even by its disorganization under the lenticels at least, thus still further accentuating the intimacy of lenticel and storage ray.

LENTICELS AND COMPOUND RAYS

In arboreal and shrubby forms the compound storage ray is only rarely met with. In the many forms examined, *Quercus* offers the only illustration. As with genera possessing aggregate rays, for example, *Alnus*, *Corylus*, *Carpinus*, it is interesting that there exist both transverse and longitudinal lenticels with the compound structures. These lenticels are small in the younger growths of *Quercus*, and as the bark is so heavy and cracked in older stems, it is difficult to find the structures at all; consequently, the investigation was mostly confined to comparatively young branches and stems. Since the lenticels are more or less insignificant, illustrations of the external views were omitted, being of little value in this connection; transverse sections, however, are shown. Fig. 12 is such a view of *Q. palustris*. The transverse lenticel is here clearly subtending a storage ray which even shows a definite curve toward the lenticel. Even at this magnification, it can be noticed that this ray was one of slow compounding. Several of those in the field are distinctly not homogeneous even in the cambial region of the wood, the light and dark rows of cells indicating the presence of both fibers and rays. The stem sectioned was nine years old, although but seven of the annual rings are visible in the photomicrograph. This means that here a nine-year old stem still has aggregate rays in process of slow compounding.

To contrast with the condition in *Q. palustris*, a corresponding section of *Q. alba* (fig. 11) is shown. Here the longitudinal lenticel, characteristic of this species, is over a curving ray as before. Although the figure does not show the inner annual rings, it may be mentioned that the time for passing through the aggregate stage to that of the fully compound ray required less than one year; in other words, the storage ray was of a much higher type, reaching its mature condition within the first annual ring. This implies a more highly evolved anatomical situation, and the fact that the elaborate storage device occurs early would necessarily mean that the structure was many times more efficient. Its metabolic activity would be greatly magnified, and this would necessitate increased respiration. The air for this purpose could most efficiently be supplied by the longitudinal lenticel. Early in the formation of rhytidome, before

the bark is so badly ridged and cracked as to make it unrecognizable, conditions such as are shown in fig. 5 are frequently found. This is an example from *Q. rubra*, which has longitudinal lenticels. Its compound rays become fully developed within the first three or four years in the specimens studied. Here vertical rows of longitudinal lenticels are clearly indicated, so much so in places that they almost resemble one greatly elongated lenticel in the proximity of the individual components. With such aerating devices as these, it is clear that the compound ray would be much more efficiently supplied than by transverse lenticels.

Although but three species have been mentioned, they are illustrative of the conditions found in the genus. Like *Quercus palustris* were found to be *Q. velutina* and *Q. coccinea*. Like *Q. alba* and *Q. rubra* were *Q. ellipsoidalis*, *Q. stellata*, *Q. macrocarpa*, and *Q. Saulii*. Thus a study of the oaks seems to indicate that the type of lenticel is not correlated with the systematic grouping of the oaks into white and black subdivisions, but one which rather implies definite anatomical differences in evolution. The lower types of storage rays, becoming compound after many years in the more primitive aggregate condition, have accompanying them the more primitive types of lenticels. In contrast, irrespective of systematic grouping, those species which quickly reach the more highly evolved and more efficient storage ray acquire the longitudinal lenticel. They also tend to become very numerous along the vertical extension of these storage rays, until longitudinal lines of them are found subtending the ray. Moreover, these longitudinal lines of lenticels may even result in fusions, until the individual lenticular fissures become continuous, giving a pseudolenticel sometimes over an inch in length.

The fact that these lenticels, irrespective of type, are invariably correlated with the rays to such an extent that the ray is still confronted by the lenticel, even when somewhat changed in its direction by growth, is difficult of interpretation unless construed in terms of gas exchange and respiration.

ROOTS OF ANGIOSPERMS

The study of the lenticels on the roots of dicotyledons shows surprising, yet not unexpected conditions. In the case of the stems,

the lenticels indicate an evolution directly parallel to the internal anatomical progress. This is not so with roots, however.

In so far as distribution is concerned, STAHL demonstrated that if lenticels were found on stems of a species, they also existed on the roots. DEVAUX agreed with this essentially, but went further, reporting them on some roots where they did not occur on stems, for example, certain herbaceous forms. The latter carefully studied the distribution of radical lenticels. In general, he found them located in pairs, one on either side of each rootlet. He proved this to be practically a universal situation, and referred to these lenticels as "basoradicellaires." He states: "Niveau d'insertion des racelles semble être un lieu d'élection spéciale pour les lenticelles de la racine." This situation the writer is able to confirm. It should be added also that they are elongated transversely. In no case of roots examined have these paired appendage lenticels been entirely absent. However, two tendencies exist which make interpretation difficult at times. There is a well known tendency for the early abortion of rootlets. In consequence, many pairs of lenticels occur with no rootlet between. When sectioned in such regions, however, in every case examined they were in the position of a rootlet trace, that is, opposite a protoxylem point. Also, lenticels in many cases abort early. Rootlets are frequently found with but one or no lenticels accompanying them. A study of younger roots nevertheless shows them to be present initially, but often they are soon lost. DEVAUX mentions these abortions, saying that they are more frequent when rootlets are crowded.

The existence of these paired lenticels is well shown in fig. 7, which is a portion of a root of *Fraxinus nigra*. At the top is a rootlet with a transverse lenticel unmistakably present on either side; at the bottom another rootlet is seen. Only one side of the lenticel appears, but on that are two transverse lenticels, side by side. Several of these structures may occur on each side of a rootlet; they get progressively smaller, however, as one gets away from the base of the appendage. This condition is a common one, especially on older roots. *Alnus incana* offers another good instance of this situation (fig. 6). At the lower right is a portion of a broken rootlet. Extending to the left from this is a chain of lenticels. To the right of

the appendage a similar condition exists, although not apparent in the illustration. In the upper part a rootlet is seen with a single lenticel visible on one side, the other again not being visible. Other instances of paired lenticels with rootlet scars between can be detected on the segment.

Many such instances might be given, for any one root is as good as another. However, these two will suffice for external views, for transverse sections are equally as instructive in this connection. Fig. 14 is a section of the root of *Sambucus canadensis*. The rootlet has just left the root; on either side is a prominent lenticel. These cases might be multiplied indefinitely, but little is to be gained thereby, since the relationship is a universal one.

The situation as illustrated here is a significant one. The similarity of the arrangement of these lenticels to the primitive condition in the stems of conifers, and the permanent condition for roots in the same group are impressive.⁵ In other words, the condition for roots at all times is that which is primitive for both stems and roots of conifers, and which is never found in angiospermous stems at any time. When this is placed along with the many other anatomical facts of equal import, one cannot with ease overlook the doctrine of the conservatism of root structures put forward by JEFFREY (6). Certainly it is true that the structure of the appendage rays in the roots does not warrant the lenticels being transverse at all times, and only related to the traces. In many cases the aggregate rays of the roots have considerable length, but in spite of this fact the lenticels are transverse. This is true in *Alnus japonica*. The aggregate appendage ray is present here, but the lenticels are still related to the rootlet, after the fashion found when but uniseriate storage structures were present, as in the conifers.

It will be remembered also that in the Coniferales cauline stomata were generally absent; this seemed to be a condition strictly accompanying the occurrence of the paired appendage lenticels. It is significant in this connection that roots are also devoid of stomatal organs, and they possess the same distribution of lenticels as in the conifers. In these two respects, therefore, the stems and roots in the conifers have their counterparts in roots of angiospermous affini-

⁵ JEFFREY, E. C., and WETMORE, R. H., Ann. Botany (Unpublished).

ties. Furthermore, it will be remembered that on coniferous stems, secondary, diffuse, transverse lenticels occurred later, obscuring the primary relationship. In this connection, attention must be called to the existence of secondary transverse lenticels on the older roots of angiosperms as well. Since the radicular appendages occur in vertical lines, the paired lenticels must be likewise arranged. These diffuse lenticels can be found scattered in regions where no rootlets could exist. DEVAUX also reported these structures. Again we have a situation similar to that in coniferous stems, although there is a difference anatomically, as previously mentioned. The ray situation of angiospermous roots warrants a more highly evolved lenticular relationship than that most suitable to the uniseriate storage elements of the conifers, yet it does not exist. The conservatism of the root seems to offer the only logical explanation.

Summary

1. Lenticels may with advantage be considered *transverse* or *longitudinal*, in accordance with the orientation of the fissure.
2. The orientation of the cauline lenticel is clearly correlated with the nature of storage ray within.
3. Forms with aggregate storage rays of primitive nature in the stem, that is, composed largely of congeries of uniseriate rays, possess transverse lenticels. In contrast, these species in which the storage aggregations consist of multiseriate units, for the most part, are found to have longitudinal lenticels. In the latter cases the pericycle usually shows a disruption under the lenticular organs. *Alnus*, *Corylus*, and *Carpinus* typify this condition.
4. Forms with compound storage rays in the stem at maturity, but in which the compounding is completed only after many years, possess the more primitive lenticels, the transverse type. Those species in which the storage ray becomes compound within a very short time, one to three years, are found to have confronting each ray one or many longitudinal lenticels. These may even be so closely approximated as to appear as a single greatly elongated structure. *Quercus* offers the only example found of this situation.
5. Roots, irrespective of internal conditions and taxonomic affinities of the species concerned, all show paired appendage lenti-

cels, the primitive type found to be characteristic of young coniferous stems and all coniferous roots, indicating the conservative nature of the root.

6. The intimate relation between the type of storage ray and the form of lenticel facilitates a satisfactory aeration of the living tissues within the stele, with the consequent maximum metabolic activity.

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EXPLANATION OF PLATES V, VI

PLATE V

FIG. 1.—Portion of branch of *Betula alba* var. *papyrifera* with transverse lenticels; $\times 1.5$.

FIG. 2.—Transverse section of transverse lenticel of *Betula populifolia*; $\times 12$.

FIG. 3.—Portions of branches of *Alnus incana* (left) and *A. japonica* (right) with transverse lenticels; $\times 1$.

FIG. 4.—Segments of stem of *Alnus rugosa* with longitudinal lenticels; $\times 1$.

FIG. 5.—Segment of branch of *Quercus rubra* with elongated vertical lenticels; $\times 1$.

FIG. 6.—Segment of root of *Alnus incana*, showing lenticels related to rootlets; $\times 1$.

FIG. 7.—Segment of root of *Fraxinus nigra*, showing relation of lenticels to rootlets; $\times 1$.

FIG. 8.—Segment of branch of *Corylus americana*, with small longitudinal lenticels appearing on transverse lenticels; $\times 1$.

PLATE VI

FIG. 9.—Portion of transverse section of *Alnus japonica*, showing relation of transverse lenticels to both aggregate and uniseriate rays; $\times 10$.

FIG. 10.—Portion of transverse section of *Alnus incana*, showing relation of lenticels to both aggregate and uniseriate rays; $\times 10$.

FIG. 11.—Portion of transverse section of *Quercus alba*, showing relation of longitudinal lenticel to compound ray; $\times 25$.

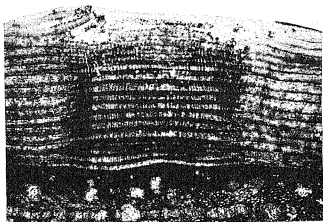
FIG. 12.—Portion of transverse section of *Quercus palustris*, showing relation of transverse lenticel to aggregate ray; $\times 25$.

FIG. 13.—Portion of transverse section of *Alnus rugosa*, indicating close relation between lenticels and aggregate rays; $\times 27$.

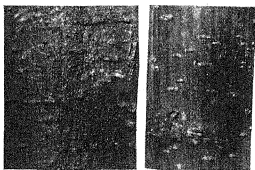
FIG. 14.—Portion of transverse section of root of *Sambucus canadensis*, with lenticel on either side of rootlet; $\times 10$.



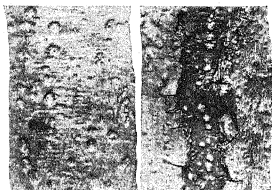
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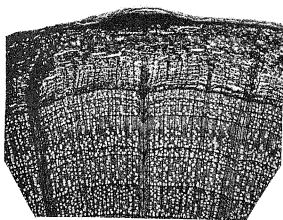


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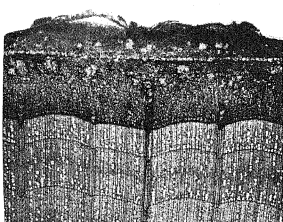


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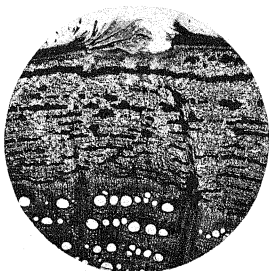




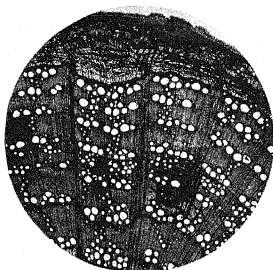
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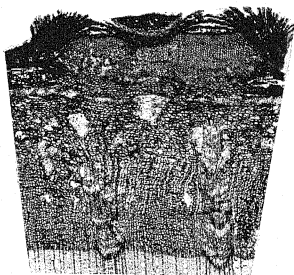
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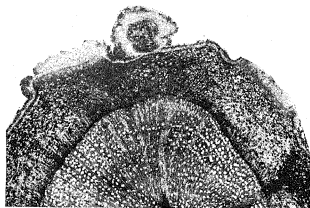
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ABSORPTION OF WATER BY BARLEY SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 350

H. S. WOLFE

(WITH ONE FIGURE)

Introduction

The phenomenon of semipermeability of non-living plant membranes seems to have been first observed just twenty years ago by GOLA. Extensive studies of delayed germination (3) apparently led him to the discovery that when seeds were allowed to imbibe water from salt solutions, only the water entered the seed itself; and the existence of a selective semipermeable layer in the seed coat was effectively demonstrated and announced (4). As this latter paper, announcing the discovery of a semipermeable seed coat membrane has hitherto escaped the notice of workers in this field, it may be of interest to note that in an examination of the seeds of 500 species belonging to some forty families, such a membrane was found to be regularly present except in the Leguminosae and in certain genera of the Cistaceae and Cruciferae. In some cases, notably that of the seed of *Cucurbita pepo*, this membrane was removed from the seed coat and used as the septum of an osmometer. GOLA's thesis was the function of the semipermeable membrane in protecting the seed from being penetrated by toxic solutes while taking in water for germination in the ground, however, and being interested in the ecological phases only, he made no quantitative studies of the rate of water intake from salt solutions through this membrane.

Two years later, BROWN (1) announced what he supposed to be the first discovery of a non-living semipermeable plant membrane, found in barley seeds. He did, however, make the first quantitative study of the phenomenon. Later (2) he amplified his data considerably, and various other workers contributed extended quantitative studies made on other seeds; notably SCHROEDER (7) on wheat, and SHULL (8) on cocklebur. No further studies on barley appeared until

1919, however, when PICKLER (5) published the results of his investigation of the diastatic activity of barley seeds and its relation to their moisture content.

PICKLER found that barley seeds soaked in a saturated solution of LiCl could take therefrom considerable quantities of water. As he is careful to point out, these results are in striking contrast to those obtained by SHULL with cocklebur, the latter demonstrating that there is practically no absorption of water from such a solution. This difference in behavior might be accounted for by the very different nature of the seed coats in the two cases, but that BROWN's work on barley is as hard to reconcile with PICKLER's as is SHULL's work on cocklebur.

BROWN did not use LiCl, but found that from a saturated solution of NaCl, barley grains would take up water, until after 48 hours an equilibrium was reached, when they had absorbed 14 per cent of their air-dry weight, and that after twelve hours they had gained only 10 per cent in weight. On the other hand, PICKLER did not approach nearly to the equilibrium condition, but stopped at the end of twelve hours, when he reported barley grains to have taken up from saturated LiCl solution 27 per cent of their air-dry weight. Now RACIBORSKI (6) gives 375 atmospheres as the osmotic pressure of a saturated NaCl solution at 20° C., and 965 atmospheres for saturated LiCl at the same temperature. PICKLER fails to state at what temperature he saturated his solution, but saturated at the temperature of the experiment (30° C.) its osmotic pressure should have been well over 1000 atmospheres. We have reported here, therefore, a greater intake of water against a resistance of 1000 atmospheres than against 375 atmospheres, and PICKLER's value agrees fairly well with that obtained by BROWN for absorption from a M-NaCl solution, with only about 40 atmospheres of osmotic pressure.

Despite this great divergence from what all previous work would lead us to expect, there was the possibility that this variety of barley was unique, for BROWN used a hulled barley, *Hordeum vulgare* var. *coerulescens*, while PICKLER employed *H. vulgare* var. *coeleste*, a hull-less variety. Although SCHROEDER and BROWN had obtained similar results with wheat and barley respectively, and the latter had shown that the removal of the glumes did not affect the behavior of his

barley, it remained to be proved definitely whether PICKLER's barley followed the rule for all other grains or was in a class by itself. Accordingly, an investigation was made of the behavior of this variety of barley in regard to absorption of water from solutions of salts, especially of LiCl.

Materials and methods

The grain used in these experiments was White Hull-less barley obtained from Vaughan's seed store in Chicago, the same variety and from the same source as that employed by PICKLER in his studies.

All experiments were conducted with media at 30° C., using an electrically controlled thermostat water bath. Saturated solutions of LiCl and NaCl were made up in stock bottles kept in the water bath, with excess of salt in the bottom of the bottles to insure saturation of the solution at the temperature of the experiment. Besides these two solutions only distilled water was used, so that whenever reference is made to LiCl or NaCl, these saturated solutions are to be understood.

The method employed was essentially that first outlined by BROWN. A known weight of grain was soaked for a definite period of time in solutions of known concentration of salts. Then the grains were removed, dried carefully on the surface and weighed, and the increase in weight calculated as percentage of the air-dry weight. A uniform procedure was adopted for this superficial drying, which is to be inferred whenever "drying" is used without qualification. The grains were rolled on dry filter paper for one minute, and then rubbed gently with a soft dry cloth for one minute. Usually twenty-five grains, carefully selected to eliminate injured ones, were employed in each determination, although in a few cases only ten grains were used.

After this superficial drying and weighing, the soaked seeds were then dried to approximately constant weight in a 50° C. vacuum oven. This is an important advance on previous methods, as it gives the actual moisture content of the soaked seeds; and when the original air-dry moisture content is known, it is possible to determine how much of the increase in weight is actually due to water intake.

While samples of grain dried in the oven simultaneously gave closely concordant values for the hygroscopic moisture in the air-dry seeds, samples taken on different days and dried in the oven for much longer periods gave values differing by as much as 1 per cent in a total of 10 per cent. To offset this source of error and to avoid the great length of time necessary for drying to absolutely constant weight (nearly two weeks), a sample of air-dry grain was put in the oven with every series of samples of soaked grain, and the air-dry value so obtained used as the factor for the calculations on the seeds of this series.

Two further refinements of technique were of value. In some cases the grains, after the stated period in the solution, were rinsed for one minute in running water after the first drying and weighing, and then dried and weighed again. In other cases the oven-dried seeds were washed in running water for one hour, and then oven-dried again. These variations in method made it possible to determine whether salts were penetrating the seed coat or remaining superficial only. All percentages recorded in the data are on the basis of air-dry weight.

Experimental results

INCREASE IN WEIGHT IN LiCl AND H_2O

Barley grains were soaked in LiCl and in water for periods of from 30 seconds to 12 hours, then dried and weighed. Table I shows the results of these experiments, and also gives for comparison the data obtained by PICKLER for some of the periods employed. The values for absorption from water are not very different in the two sets of data, his being consistently a slightly higher percentage, but the absorption from LiCl is totally dissimilar. Fig. 1 presents these data graphically and more strikingly. The LiCl curve for the data of these experiments flattens out very early, with a maximum at about 7.5 per cent, and has reached only 9 per cent after 200 hours, whereas in PICKLER's data the LiCl curve is still rising rapidly at the end of twelve hours, and has already reached 27 per cent. This curve is quite similar to that given by BROWN for absorption from M-NaCl .

TABLE I

GAIN IN WEIGHT OF BARLEY SEEDS IN WATER AND IN LiCl SOLUTION FOR PERIODS UP TO 12 HOURS; PICKLER'S FIGURES ALSO GIVEN FOR COMPARISON

TIME OF SOAKING	GAIN IN PERCENTAGE OF AIR-DRY WEIGHT IN		CALCULATED FROM PICKLER'S DATA	
	LiCl	H ₂ O		
30 seconds.....	0.72	3.57	GAIN IN PERCENTAGE OF AIR-DRY WEIGHT IN	
1 minute.....	0.79	3.45		
2 minutes.....	0.81	4.94		
5 minutes.....	1.61	5.61		
10 minutes.....	2.38	7.86		
30 minutes.....	3.50	10.46	LiCl	H ₂ O
60 minutes.....	4.00	12.29	14.85	21.27
2 hours.....	5.95	18.57	19.00	29.17
4 hours.....	6.65	25.07	21.21	34.18
6 hours.....	6.89	22.65	23.86	38.95
8 hours.....	6.65	31.35	25.75	43.72
10 hours.....	7.53	34.44	26.85	46.25
12 hours.....	7.29	39.33		

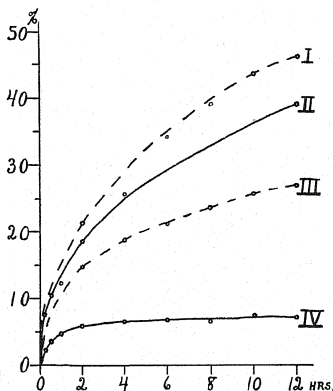


FIG. 1.—Curves showing increase in weight of barley grains in water (I and II) and in LiCl (III and IV); broken line curves from PICKLER'S data; abscissae, time of soaking seeds; ordinates, gain in weight of seeds as percentage of air-dry weight.

NATURE OF INCREASE IN WEIGHT

Having determined the amount of increase in weight on soaking in LiCl, the question arose as to whether this were all due to the absorption of water. Preliminary experiments indicated that such was not the case, and further experimentation gave the results summarized in table II. Seeds were soaked, dried and weighed, then oven-dried and weighed; and some were then washed, oven-dried and weighed again. The air-dry factor for the final oven drying was 11 per cent for all except the 200 hour sample, for which it was 11.5 per cent.

These data show that by no means all of the increase of the weight of seeds soaked in LiCl is due to intake of water. The water intake has practically stopped by the end of the second hour, for even after 200 hours the increase in water content was hardly greater than at the end of two hours. The rest of the gain in weight is due to LiCl, which is only superficially absorbed, however, for it can all be removed by an hour's washing in running water. There appears to be a slight increase in absorption of LiCl with time, but as there is only one determination for 200 hours, no very definite statement can be made. The data show a steady increase in LiCl absorption for the first twelve hours, these five samples having been run simultaneously. The next four 12-hour determinations give variable values, however, but all within 0.5 per cent of the mean. It should be stated that variations of less than 1 per cent are not to be considered significant, as this is within the limit of experimental error for the drying of the seeds, and for the variation in different lots of seeds. The figures on which conclusions are based are not only concordant to less than 1 per cent, however, but are themselves of an order much larger than this.

Attention is called to the close correlation between the air-dry moisture content, as determined on several different samples of seeds, and the sum of the amount of loss below air-dry on oven drying plus the amount of gain not accounted for as water. This, of course, simply confirms the values obtained under the conditions of oven drying in these cases for the air-dry moisture content of the samples, but such confirmation is important. Of even greater interest is the correlation between the gain in weight unaccounted for as

TABLE II
ABSORPTION OF WATER AND SALT FROM SATURATED SOLUTIONS OF LiCl BY BARLEY SEEDS

HOURS IN LiCl	WEIGHT (G.M.) AIR-DRY	WEIGHT (G.M.) OVER-DRY SOAKED	WEIGHT (G.M.) OVER-DRY SEEDS TIME	WEIGHT (G.M.) OVER-DRY SEEDS TIME	PERCENT- AGE GAIN IN WEIGHT IN SOLUTION	TOTAL H_2O IN SEEDS SOAKED	TOTAL H_2O IN SEEDS AIR-DRY	PERCENT- AGE GAIN IN WEIGHT DUE TO H_2O	PERCENT- AGE LOSS BELOW AIR-DRY ON FIRST DRYING	PERCENT- AGE LOSS ON SECOND DRYING	SAME COR- RECTED FOR AIR-DRY H_2O	PERCENT- AGE LOSS IN WEIGHT DUE TO WASHING
1.....	1.0520	1.1035	0.9690	4.90	12.78	10.50	2.30	7.90
2.....	1.0250	1.0860	0.9425	5.95	14.00	10.50	3.50	8.00
4.....	1.1380	1.2350	1.0790	6.65	13.50	10.50	3.00	6.85
10.....	1.0620	1.1415	0.9000	7.53	14.27	10.50	3.75	6.74
12.....	1.0570	1.1340	0.9880	7.29	13.81	10.50	3.30	6.52
12.....	1.1205	1.2030	1.0415	0.9965	7.36	14.40	10.50	3.90	7.05	11.07	10.57	3.52
12.....	1.1495	1.2300	1.0660	1.0160	7.00	14.26	10.50	3.80	7.26	11.61	11.11	3.85
12.....	1.1750	1.2585	1.0930	1.0455	7.10	14.10	10.50	3.60	7.00	11.02	10.52	3.52
12.....	1.1830	1.2600	1.0975	1.0470	6.50	13.75	10.50	3.25	7.23	11.50	11.00	3.77
200.....	2.2045	2.4700	2.1605	1.9900	9.10	13.70	10.50	3.20	4.59	12.10	10.00	6.00

water, and the loss in weight on washing after the first oven drying. This loss in weight is slightly greater than the theoretical in three of the five cases, probably indicating that there has been some leaching of the salts normally present in the seeds, but the closeness of the correlation amply justifies the conclusion that very little of the loss in weight on washing is to be explained thus.

A modification of this procedure was used at first, and yielded very interesting results, although not definitive. The seeds were soaked in LiCl, dried and weighed, rinsed in running water for 15-60 seconds, and dried and weighed again. The whole process, from taking the seeds out of the solution to the end of the final weighing, occupied less than 10 minutes, so that loss by evaporation was negligible. As there was no oven drying, no data were obtained regarding the absolute amounts of water in the seeds, but it was at once observed that with increase of rinsing time up to 60 seconds, decrease in seed weight followed, despite the fact that seeds were absorbing water rapidly during the rinsing. With periods of rinsing longer than 60 seconds, absorption of water was greater than loss of salt.

In table III are given the results of two sets of experiments of this kind, in which the seeds had been soaked for 12 hours and rinsed for 60 seconds. As a control, to determine the increase in water content due to rinsing, there is used one of the samples from table II which happened to be almost exactly the average for the five 12-hour samples. In the present experiment two samples were washed again after oven drying, and the other two were dried with them a second time without washing, so as to give a correction factor for any greater drying the second time. The factor was 0.6 per cent in both cases, and it is duly subtracted in the proper column.

These data show that nearly all of the absorbed LiCl is rinsed off in 60 seconds when the seeds are still moist. No attempt was made to determine the minimum time for complete removal of absorbed salt, as the only important point was the possibility of completely removing it by fairly brief washing. There is to be noted a startling agreement in the values given for the total absorption of water by the seeds from LiCl. This unusual concordance is largely due to the use of a common factor (14.1 per cent) for determining

TABLE III
EFFECT OF BRIEF RINSING ON WATER AND SALT ABSORPTION

SAMPLE	a	b	c = a - b	d	e	f	g = d - f	h = b - g	i = e + h	j	k	l	m = l - j	n = h - i	o = a - n
	PERCENTAGE GAIN IN WEIGHT IN SOLUTION	PERCENTAGE GAIN IN WEIGHT AFTER RINSING	PERCENTAGE LOSS IN WEIGHT ON RINSING	TOTAL PERCENTAGE H ₂ O IN SEEDS SOAKED	PERCENTAGE INCREASE IN H ₂ O OVER UNRINSSED	TOTAL PERCENTAGE H ₂ O IN SEEDS AIR-DRY	PERCENTAGE GAIN IN WEIGHT DUE TO H ₂ O	PERCENTAGE GAIN IN WEIGHT NOT DUE TO H ₂ O	TOTAL PERCENTAGE LOSS BY RINSING	PERCENTAGE LOSS BELOW AIR-DRY ON FIRST OVEN-DRYING	PERCENTAGE SAME ON SECOND OVEN-DRYING	PERCENTAGE LAST CORRECTED FOR AIR-DRY H ₂ O	PERCENTAGE LOSS IN WEIGHT FROM WASHING	PERCENTAGE L ₂ CL ₂ ABSORBED IN 12 HOURS	PERCENTAGE H ₂ O ABSORBED IN 12 HOURS
1.....	8.27	7.72	0.55	17.10	3.00	10.50	6.60	1.10	3.55	9.38	11.44	10.84	1.45	4.65	3.60
2.....	8.21	7.71	0.50	17.00	2.90	10.50	6.50	1.20	3.40	9.27	(9.85)	(6.06)	4.00	3.00
3.....	7.55	6.96	0.60	16.58	2.50	10.50	6.10	0.85	3.10	9.03	11.43	10.83	1.20	3.95	3.00
4.....	7.69	6.94	0.75	16.71	2.60	10.50	6.20	0.75	3.35	9.77	(10.35)	(6.60)	4.10	3.00
Control.....	7.11	14.10	10.50	3.60	3.50	7.00	11.00	10.50	3.50	3.50	3.60

the increase of water in rinsed over unrinsed seeds, as previously explained, but 3.6 per cent is the average figure for the five 12-hour samples of table II, and so undoubtedly is the correct average for the samples of table III, although probably inaccurate for any one of them.

BEHAVIOR OF WATER-SOAKED SEEDS IN LiCl AND NaCl

As a final test, grains of barley were soaked in distilled water for 12 hours, dried and weighed, and at once put into saturated LiCl solution containing excess of salt. After 12 hours the grains were removed, dried and weighed again. A second pair of samples was treated similarly, but with NaCl instead of LiCl. Table IV gives the results of these experiments.

TABLE IV

LOSS OF WEIGHT OF WATER-SOAKED BARLEY GRAINS IN LiCl AND NaCl

SALT SOLUTION USED	AIR-DRY WEIGHT OF SEEDS (GM.)	WEIGHT (GM.) AFTER 12 HOURS IN H ₂ O	PERCENTAGE GAIN IN WEIGHT	WEIGHT (GM.) AFTER 12 HOURS IN SALT	PERCENTAGE GAIN OVER AIR-DRY	PERCENTAGE LOSS OF WEIGHT IN SALT
Saturated LiCl.....	1.1410 1.2015	1.5790 1.6725	38.39 39.20	1.3365 1.4175	17.14 17.98	21.25 21.22
Saturated NaCl.....	1.0555 1.2570	1.4649 1.7625	38.79 40.57	1.2910 1.5510	22.31 23.40	16.48 17.17

Of course the seeds were far from being in equilibrium with the solution at the end of 12 hours, but in LiCl the water content has dropped from an initial 39 per cent to below 18 per cent, and in NaCl to below 23 per cent. This takes no account of the amount of this gain over air-dry weight which is due to salt absorption, and as the previous data have shown this to be about 4 per cent for LiCl, it is seen that the water content has really dropped to near 14 per cent. Experiments not here reported have shown that after 12 hours in NaCl, barley grains have increased about 11 per cent over their air-dry weight, and there seems little reason to doubt that with longer periods of time an equilibrium would be reached under these conditions at about 8 per cent for seeds in LiCl and 11 per cent for seeds in NaCl. The point of importance, however, is the impossibility of barley grains increasing to 27 per cent over their air-dry weight by

absorption of water from saturated LiCl solution, as PICKLER reported, or even from saturated NaCl in 12 hours; for when put into these solutions with a greater water content than this, they lost within 12 hours until they had only half as much water in them in the LiCl sample, and considerably less than 27 per cent in the NaCl sample.

Discussion

The results of the present investigation are so different from those obtained by PICKLER that comparison is almost impossible. There seems to be no possible explanation of this unconformity other than that he used by mistake some other salt instead of LiCl (although this is almost unthinkable). The absorption curve of his data is unmistakably that for a solution of low osmotic pressure, and his germination studies indicate that his seeds really absorbed from solution considerable quantities of water.

It was thought at first that his anomalous results might be due to employment of a solution saturated below the temperature of the experiment, but experiments conducted under such conditions showed only insignificantly greater increases in weight of seeds. Also the very form of the absorption curve precludes the possibility of the solution having been nearly saturated. It is unfortunate that PICKLER did not try cocklebur seeds when he found barley exhibiting such different behavior, for he certainly would have found a large intake of water from his solutions by cockleburs also.

The present study leaves no doubt that air-dry barley grains are not able to exert such tremendous internal imbibitional force as would be indicated by PICKLER's observation of 27 per cent intake of water in 12 hours from LiCl against an osmotic pressure of 1000 atmospheres. The seeds, however, are able to take in about 3.6 per cent of water from such a solution in 12 hours at 30° C., whereas SHULL reported no absorption by cocklebur seeds under the same conditions, except that he used room temperature which would affect only the rate at which equilibrium was attained, so far as we are concerned. This lack of conformity may mean that barley seeds have a greater imbibitional force when air-dry than have cocklebur seeds, but the structure of the seed coats must be taken into consideration.

A median section of the cocklebur seed shows that it consists of an outer epidermal layer, a middle stratum of four or five layers, and the inner layer representing the nucellar epidermis. This last is the semipermeable layer proper, as SHULL demonstrated, but the middle layers are greatly compressed and have some osmotic properties. The outer layer represents nearly half the thickness of the entire seed coat, and is quite chaffy and fragile. There is no doubt that in his efforts to remove all surplus water and salt, SHULL rubbed off this outer layer in the process of drying the seeds. There would be left very little of the seed coat outside of the semipermeable layer to hold water, and if the membrane were truly semipermeable to LiCl, and that salt in saturated solution equaled or exceeded the internal attractive forces of the seed, then no increase in weight would be observed.

Barley grains, on the other hand, are well known to be not seeds at all, but fruits with pericarps of firm texture. In the case of the hull-less variety used in these experiments, there are about ten layers of cells external to the semipermeable layer, which both GOLA and BROWN showed independently to be the single remaining layer of the nucellus, its epidermis. The degree of rubbing which would remove the outer layer of the cocklebur seed coat would hardly affect that of the barley, and its seed coat is shown by microscopic measurement to have three times the thickness of the portion of the cocklebur seed coat left after the outer layer has been rubbed off. The quantity of water which these external layers of the barley coat can hold is manifestly not at all negligible. Furthermore, the barley grain has a deep longitudinal suture, which holds water very tenaciously even with careful drying, while the cocklebur seed is quite smooth of surface. It is therefore entirely reasonable that barley grains should show greater increase in weight than cocklebur seeds, in solutions of the same osmotic pressure, and such greater gain does not necessarily indicate a greater internal attractive force for water by barley.

The most important point which this study brings out is that gain in weight in solution is not an indication that seeds are withdrawing water from the solution. Previous investigators seem to have overlooked the possibility of salt absorption, and have assumed that all gain in weight was due to intake of water. As a matter of

fact, neither BROWN nor PICKLER had any data on the amount of water imbibed, but only as to the combined absorption of water and salt. Whereas in the cocklebur seeds there was no absorption of either water or salt, for reasons already given, in the barley grains considerable salt can be held by the many layers external to the nucellus. A saturated solution of LiCl is very viscous, and clings closely to the surface of the barley grains, as well as accumulating in the suture.

From the fact that there seems to be no increase in the water absorption after the second hour, while the salt absorption seems to increase slowly though steadily with time, the conclusion may be drawn that there is a precipitation of the salt in these superficial layers of cells where the salt and water are absorbed. The data are very meager for determining this point, but in substantiation may be cited that after 12 hours in LiCl a greater weight of salt has been absorbed than of water, although in a given volume of the solution there is a greater weight of water than of salt, 85 per cent being the weight solubility of LiCl at 30° C. The ease with which the salt can all be washed out is strong proof that it is really all located in these superficial layers external to the semipermeable one, and, furthermore, if salt penetrated deeper, then water would be taken in deeper also, whereas the data show that water absorption quickly reaches a maximum and remains constant thereafter.

When barley grains which had been soaked for 12 hours in LiCl were cut open, their endosperm was found to be quite dry still. Even after 200 hours' soaking this was true. Also, after 12 hours' soaking in LiCl the grains still retained their original pearly luster, whereas 10 minutes in water was sufficient to cause the loss of this, the acquisition of a certain translucence in its stead, and a softening of the endosperm. This opacity of aspect seems to be lost as soon as the endosperm begins to take in water. All these considerations make it seem evident that barley grains are not able to extract water through their semipermeable membranes against the resistance offered by saturated solutions of LiCl, although the layers external to this membrane may absorb considerable quantities of water and of salt.

It is interesting to note that BROWN found that barley grains could not absorb enough water from 36 per cent H_2SO_4 to moisten

the endosperm perceptibly. Although still as brittle as ever and apparently air-dry, yet there is probably some slight increase in moisture content, since SHULL (9) found in other seeds of the same family that when they were exposed over 36 per cent H_2SO_4 , there was a gain of about 3 per cent in weight. The boiling point of such acid is about 115° and its freezing point -63°C . Calculations of the osmotic pressure from these colligative properties of the vapor pressure agree at about 650 atmospheres. We have, therefore, no intake of water against 1000 atmospheres pressure, not enough to cause any apparent change in endosperm texture or appearance against 650 atmospheres, but quite appreciable intake, with evident endosperm moistening, against the 375 atmospheres of NaCl .

Summary

1. Barley grains which have been soaked in saturated solutions of LiCl for periods of from 30 seconds up to 12 hours show a curve of increase in weight which has practically reached its maximum of 7.5 per cent at the end of the first 2 hours.

2. When such grains, after 12 hours' soaking, are oven-dried, they fail by 3-5 per cent to lose weight equivalent to their gain in weight plus their original air-dry content of moisture, that is, they have either absorbed or adsorbed some salt.

3. When these oven-dried grains are washed for an hour in running water and are then oven-dried again, they show a loss in weight of about 3-5 per cent over the first oven-dry weight, that is, the salt is so located that it is readily washed off.

4. Grains which after 12 hours in LiCl are rinsed for 60 seconds in running water before oven-drying, fail by only 0.5-1.0 per cent to dry to the theoretical oven-dry weight, and on further washing for an hour lose about that much more weight.

5. The average of eight determinations gives 3.6 per cent of water absorbed in 12 hours from saturated LiCl at 30°C ., the rest of the observed weight being due to absorbed salt.

6. More water is absorbed by barley grains from distilled water in 2 minutes than from LiCl in 12 hours, and there is more gain in weight in 12 minutes in distilled water than in 12 hours in LiCl .

7. The increase in weight of barley grains soaked in LiCl is due to absorption of water and salt by the numerous layers of the grain

external to the semipermeable layer, and not to penetration of either water or salt into the interior of the grain.

8. The data obtained in these experiments agree quite well with those obtained by BROWN, SCHROEDER, and SHULL with other seeds, but show no agreement with those given by PICKLER for the same variety as was here used. No explanation seems possible for this unconformity, except that perhaps PICKLER did not use LiCl as he supposed he did, and such an explanation is hardly entertainable.

9. Attention is called to the error commonly made of assuming that the increase in weight of seeds placed in salt solutions, especially saturated solutions of great viscosity, is due entirely to intake of water. Actually it may be that as much as half of this gain in weight is due to absorbed salt.

It is a pleasure to express my obligation to Professor CHARLES A. SHULL, who first called my attention to the anomalous findings which suggested this study, and who has been of assistance throughout the investigation.

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BRIEFER ARTICLES

CORRUGATED ALUMINUM SHEETS FOR THE BOTANIST'S PRESS

(WITH TWO FIGURES)

For several years past corrugated pasteboards between the felt driers in the plant press, together with the use of artificial heat, usually from a kerosene stove or camp fire, have proved a great boon to the collecting botanist, especially when sunshine is rare and rain constant. Such devices have been admirably described by HITCHCOCK,¹ and little can be added to his discussion. I have used equipment much like that of HITCHCOCK's with great satisfaction, being able to dry a day's collection over night, even though the plants were wet with rain and the driers also soaked. The corrugated pasteboards are bulky and comparatively heavy, however, and I therefore venture to describe the following lighter and more compact press equipment.

Instead of the corrugated pasteboard, aluminum sheets cut 30.5×61 cm. and 0.15 mm. thick are corrugated by running grooves 4 mm. deep and 13 mm. wide across them. The result, after corrugating is completed, is a sheet 30.5×38 cm., approximately the dimension of the usual drier.

Specimens are placed as usual in folders of newspaper or other similar paper. The folders may then be placed between two ordinary driers and these between two sheets of corrugated aluminum. Thus the normal sequence is drier, folder, drier, aluminum. The thinnest botanical drier that gives sufficient stability is best.

In practice I find that often half the number of driers just suggested can be omitted, and that three folders can be placed between two aluminum sheets, making the sequence drier, 3 folders, aluminum. Whether the specimen next to the aluminum will show any corrugation or not depends upon its nature. Often there is no apparent effect, and almost always it is very slight. If the kind of specimens permit it all the driers may be omitted, therefore, only the folders and aluminum sheets being used. Familiarity with the nature of the plants collected will readily enable the collector to decide whether, and to what extent, felt driers are necessary. The gain by decreasing the size of the pack enables the handling of more

¹ HITCHCOCK, A. S., Jour. N.Y. Bot. Gard. 21: 129-137. 1920. ———, Methods of descriptive systematic botany. 1925.

than three times as many folders. The aluminum, doubtless due to its capacity to conduct heat, results in much more rapid drying than by the use of corrugated pasteboards.

Instead of the usual wire frames for each side of the pack, aluminum frames can be used, with a great saving of weight. Such aluminum cast frames are shown in fig. 1. These measure 30.5×46 cm., and 6 mm. in section.

To support the press over the heater, a table made by screwing aluminum legs into the four corners of a press side, constructed with special

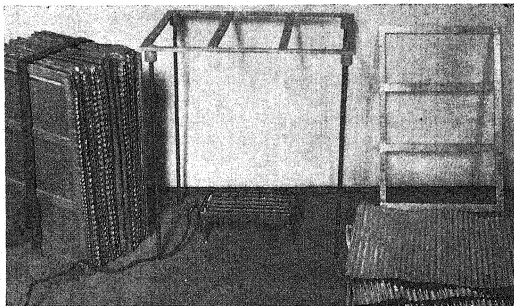


FIG. 1.—Press equipment: pack made up ready for drying, aluminum table with iron legs, electric heater, aluminum press ends, and three corrugated aluminum driers.

corners to receive the legs, is an improvement on temporary camp supports. Two lengths of legs should be carried, one set 30 cm. long to use with electrical heat, such as the electric toaster shown in fig. 1, and one set 48 cm. long to use over a kerosene heater. To secure the necessary pressure on the pack, a trunk strap of suitable length is best.

Electricity is the most useful heat, and is now available so generally that provision for its use should be made. An electric toaster with removable legs packs with economy of space.

Kerosene may be used when electricity is not available, employing a small two-wick stove. Still better perhaps is the plan of having made two kerosene reservoirs (fig. 2), about 29×6 cm., and fitting each with a burner taken from an ordinary kerosene stove. These pack economically and give a large kerosene capacity. In some instances it may be ad-

visible to substitute canned heat for kerosene, but I cannot yet speak from experience regarding its use.

The pack when made up is placed upon the table with the corrugations in a vertical position. A skirt of khaki is then placed around the pack, its bottom reaching to the floor, its top sufficiently secured in place

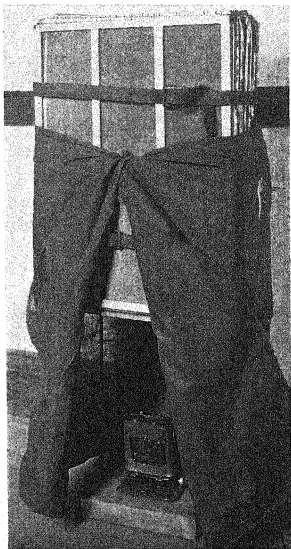


FIG. 2.—Drier set up over kerosene stove with specially constructed reservoir

by tucking the edge in the pack. A second pack also with a skirt may be placed on top of the first (fig. 2). The plants in this second pack will be considerably dried, although of course not so thoroughly as those in the lower pack.

The convenience of the outfit is such that it can be set up in less than five minutes and packed away for transport in a similar time.—F. L. STEVENS, *University of Illinois, Urbana, Ill.*

CURRENT LITERATURE

BOOK REVIEWS

Revaluation of biological concepts

The relativity and quantum theories have necessitated the revaluation by scientists and philosophers of the data and concepts of the physical sciences. Naturally the biological sciences could not very long escape a similar revaluation. Because of the prestige of the physical sciences, biology "apes the manner of physics" and it has been "orthodox to hold that there is nothing in biology but what is physical mechanism under somewhat complex circumstances." Consequently a revaluation of the data and concepts of the biological sciences is due, not only because revaluation is in the air, but even more because the philosophical foundations on which biology build its concepts are giving way. This situation is very ably and clearly discussed by WHITEHEAD¹ in *Science and the modern world*, from which the quotations are taken. WHITEHEAD further states: "It cannot be too clearly understood that the various physical laws which appear to apply to the behavior of atoms are not mutually consistent as at present formulated. The appeal to mechanism on behalf of biology was in its origin an appeal to the well-attested self-consistent physical concepts as expressing the basis of all natural phenomena. But at present there is no such system of concepts." WHITEHEAD also criticizes the current vitalisms for accepting mechanism based on materialism and merely introducing an additional vital factor. He contends that science is taking on a new aspect which is neither purely physical nor purely biological; it is becoming the study of organisms. Biologists will find this an interesting and stimulating book in spite of its heavy flavoring with metaphysics.

ELDRIDGE² subjects the data of biology to a logical, philosophical, and metaphysical analysis. Such problems as inheritance of acquired characters, variation, and heredity receive exhaustive and critical treatment. The author contends that the phenomena of organization and regulation as we see them in organisms defy attempts to fit them into the concepts of materialistic mechanism which have dominated the philosophy of science. The author in the light of logic and philosophy prefers to choose the path of the vitalists. The vitalistic conceptions of BERGSON, DRIESCH, and others, however, are subjected to the same critical analysis applied to the mechanistic conceptions. The author's vitalism

¹ WHITEHEAD, A. N., *Science and the modern world* (Lowell Lectures 1925). Pp. xi+296. Macmillan Co. 1925.

² ELDRIDGE, SEBA, *The organization of life*. pp. xv+470. Thomas Y. Crowell Co. 1925.

is one which permits functional activity and experience to affect the "organizational factors." Although the entelechies advocated can apparently be of little aid at present to the experimental biologist, he should not let this fact prejudice him against the book. Its value lies in its critical analysis of the data and concepts of biology. ELDRIDGE's book carries an introduction by H. S. JENNINGS which is a thorough-going and fair review. The following quotations are taken from the introduction. "The author has surveyed the chief doctrines of vitalism, mechanism and their congeners, with the arguments offered for and against them, and sets these forth clearly. He prepares for us a map of this wilderness, marking the paths leading to different destinations; and putting up at each fork a sign calling attention to the different fates in store for us according as we take the path to the right or that to the left. . . . The map maker gives us . . . his own preferred route. . . . His conclusions lie in the opposite direction from those reached by the present writer over *his* preferred path of radically experimental analysis. For the serious student, intending to determine his own route, the map is more important than the recommendation as to preferable roads." "The work, too, is a concrete example under the conviction that . . . in addition to observation and experiment, science has a need of far reaching inferential ratiocination for its proper working out. . . . Beside its use as a compendium of the matters to be weighed and considered in theoretical biology, it gives opportunity for judging the fruits of such extensive ratiocinative treatment of scientific data."—G. K. K. LINK.

Chemical action of ultraviolet rays

Ultraviolet radiations have been found capable of producing many different kinds of chemical reactions, some of which are of unusual interest to the physiologist and physiological chemist. A very extensive literature has grown up, in physical, chemical, and electrical journals, as well as in medical and biological publications. ELLIS and WELLS³ have summarized the information about ultraviolet rays in book form. The first four chapters may be considered introductory. They deal with the nature of ultraviolet, sources of the rays, the various metal vapor lamps used in artificial production of these rays, and the protective screens, filters, and glasses useful to the investigator. The six succeeding chapters deal primarily with the chemical effects of ultraviolet, the photochemical mechanism, behavior of gases in presence of ultraviolet radiations, photochemical and photolytic reactions, and the influence of these short rays in halogenation reactions. The next four chapters take up the biological reactions, photosynthesis, sterilization by ultraviolet, biological effects on proteins, enzymes, antibodies, chemistry and histology of blood, metabolism, avitaminosis, etc., and therapeutic applications. The final chapter deals with miscellaneous applications of ultraviolet rays, particularly in the industries.

³ ELLIS, C., and WELLS, A. A., *The chemical action of ultraviolet rays*. 8vo. pp. 362. *figs. 85*. New York: Chemical Catalog Co. 1925.

Plant physiologists will be most interested in the discussion of photosynthesis, photochemical reactions, sterilization, and biological effects. At the close of the last mentioned chapter, there is a very brief summary of the influence of ultraviolet radiations on germination of seeds and the growth of plants by POPP, who is engaged in extensive investigations of this subject.

The reviewer feels that the authors have possibly not been sufficiently critical toward the interpretations which some investigators have placed upon their work. In some cases where the original sources are hypothetical and speculative to a high degree, these authors give the impression that their claims are practically proved. One must therefore read carefully, and with some reference to the original sources, if he would avoid misjudging the present condition of our knowledge with reference to the effects of light upon chemical metabolism, particularly synthesis, in plants.

The subject had grown to the point where such a book was needed, and it will find a welcome among those who are interested in experimentation with radiations beyond the range of visibility.—C. A. SHULL.

Physiological basis of drought-resistance

The physiological side of drought-resistance has received critical consideration by MAXIMOW,⁴ who has drawn together many interesting observations on absorption, transpiration, and the problems of water balance and water deficit in the plant. Unfortunately it is published in Russian, but it includes a brief abstract of about 15 pages in English at the close of the discussion.

The book is divided into three main sections, the first of which consists of four chapters dealing with the absorption of water, the laws governing intake, and the conditions under which water enters the plant. The first chapter considers the suction power idea of URSPRUNG, the influence of water deficit on the growth of cells, anatomical details for reduction of transpiration, and the necessity of the transpiration stream. In chapter II, the author accepts the idea of active and passive absorption, the latter brought about by transmission of suction power developed in the leaves to the root cells through the cohesion of water in the tracheae. The third chapter takes up the soil conditions, its water retaining power, wilting coefficients, and water transporting power. The last chapter of this section deals with the influences of environment, low temperature, bog xerophytism, etc.

The second section, of six chapters, deals with water elimination in liquid and gaseous form, physics of transpiration, stomatal movements, stomatal control of transpiration, and physics of the transpiration stream.

The last half of the book considers the water balance, in five chapters. The normal water balance and conditions causing deficit are considered in chapter XI, while the following chapter takes up the peculiarities of groups of plants

⁴ MAXIMOW, N. A., The physiological basis of drought-resistance of plants. 8vo. pp. 436. figs. 67. Inst. Applied Bot. and Plant Breeding. Leningrad. 1926.

that differ in water relations, succulents, desert ephemeres, and ordinary xerophytes. Chapter XIII considers the relations between drought resistance and certain physiological and anatomical peculiarities of plants. Little correlation is found between drought resistance and intensity of transpiration, or between transpiration and dry weight production. Chapter XIV examines the problems of xeromorphy from new points of view. The fundamental trait of xeromorphy is said to be "increased density of nervation, which points to an augmented water supply, and an increased amount of stomata pointing to a high transpiration." Xeromorphic plants are not characterized by restricted water relations, but by plastic ones. They possess the capacity to endure extreme wilting without injury, and to carry on more intense vital functioning in times of water abundance.

The final chapter applies the new conceptions of xeromorphy to the problems of breeding drought-resistant plants. Endurance is conditioned mainly by protoplasmic behavior and the accumulation of substances which protect the protoplasm from coagulation under wilting.

MAXIMOW writes that an attempt to have his work translated into English is being made. It is hoped that this may be accomplished, as the work constitutes a stimulating summary of this important physiological agricultural problem.—C. A. SHULL.

The phylogenetic method in taxonomy

HALL and CLEMENTS⁵ have published a paper suggesting some fundamental changes in the preparation of taxonomic monographs. The new method is illustrated by monographs of the North American species of *Artemisia*, *Chrysothamnus*, and *Atriplex*. Instead of merely grouping the species on the basis of resemblances and differences, the attempt is made to work out the course of evolution they represent. The results are presented in diagrams, indicating not only the general direction of evolution, but also the characters that have undergone change. Some of the conclusions are as follows.

Artemisia is supposed to have developed from an ancestor resembling *Chrysanthemum* and *Tanacetum*, the most primitive section being *Abrotanum*. *Chrysothamnus* is thought to have developed from a hypothetical group close to a section of *Haplopappus*. In *Atriplex* the probable primitive condition is described, the species most nearly representing it being *A. hortensis*. The authors have drawn their conclusions not only from the ordinary taxonomic characters of these genera, but also from their ecological relationships, as obtained from extensive field work. They urge the inadequacy of herbarium studies unaccompanied by investigations in the field.

The troublesome question of nomenclature is also considered, and the attitude of the authors may be inferred from the following quotation: "There has

⁵ HALL, H. M., and CLEMENTS, F. E., The phylogenetic method in taxonomy. Publ. Carnegie Inst. no. 326. pp. 355. pls. 58. 1923.

been no hesitation in selecting names from those valid under the American Code when these are preferable, and no compunction has been felt in using names sanctioned by neither code when this has been found to be in the interests of usefulness." This of course is carrying nomenclature back to the days preceding any code and destroying its stability.

Another view advanced relates to the limitations of genera and species. Data are given showing the rapid increase in the number of recognized species during the last few years, and also in the number of recognized genera. HALL and CLEMENTS advocate a return to the old concepts of genera and species, and a checking of the present tendency to extreme segregation. They illustrate this point by contrasting their treatment of *Artemisia* and *Atriplex* with that of the *North American flora*. They accept 29 species of *Artemisia*, while in the other publication these are presented as 125 species in 4 genera. In *Atriplex* they recognize 47 species, while in the other publication these are presented as 106 species in 2 genera. Much of this difference is doubtless due to the emphasis which HALL and CLEMENTS place upon the effect of environmental conditions. These different opinions as to generic and specific limitations can only be reconciled by field study and breeding experiments. Of course the species concept must be considered also, HALL and CLEMENTS urging a return to the larger concept of the older taxonomy.—J. M. C.

Families of flowering plants

Revisions of systems of classification are constantly appearing, and often attract little attention beyond the limits of the small groups immediately favorable or decidedly opposed to the changes they advocate. It seems safe to predict, however, that the present volume⁶ will be more widely noticed and will stimulate more interest in the possible relationships between families of angiosperms. It is certainly the most noteworthy attempt to solve the puzzle of phylogenetic lines that has been attempted since the system connected with the names of ENGLER and PRANTL appeared.

Those who regarded the treatment of the Amentiferae and that of the monocotyledons as presented by ENGLER and PRANTL as far from satisfactory, may be pleased with the position given these groups in this new system. HUTCHINSON has ventured to develop double phylogenetic lines, with many branches, having the Ranales and the Magnoliaceae as their respective starting points. From the former has come an herbaceous complex which includes the monocotyledons and many of the families usually placed in the phylum sprung from the Ranunculaceae and culminating in the Asterales (Compositae), Campanales and Lamiales (Labiatae).

From the latter has come a complex that includes the greater number of

⁶HUTCHINSON, J., The families of flowering plants. I. Dicotyledons. Arranged according to a new system based on their probable phylogeny. 8vo. pp. xiv+328. figs. 264. London: Macmillan & Co. 1926.

the more strictly arboreal families. This gives emphasis to the fact that trees and shrubs are at least as primitive as herbs. In both groups petaliferous flowers are regarded as preceding apetalous ones, and bisexual as preceding unisexual flowers. This has resulted in giving the Amentiferae a position as a lateral branch decidedly more specialized than the Magnoliaceae from which they are descended through the Rosales.

The proposed system will decidedly increase the number of plant families. Thus while ENGLER and PRANTL in their *Pflanzenfamilien* have some 280 families of flowering plants, including gymnosperms, HUTCHINSON, in this his first volume, makes the dicotyledons include 264 families. He claims that not only are these families composed of more homogeneous material, but also that they are grouped into smaller orders that show closer general relationships. In this grouping resemblances are emphasized rather than differences.

Among some of the features of this volume that should recommend it to the general botanist, if not to the taxonomist, are its introductory discussion of the principles of classification, its key to the families of dicotyledons, its numerous sketch maps showing the distribution of different genera and families, and the originality evident in the drawings illustrating the structure of the flowers of the various families.

The volume is dedicated to the memory of GEORGE BENTHAM and JOSEPH DALTON HOOKER, authors of the *Genera Plantarum*, whose views have influenced the present author in his phylogenetic arrangement.

The printers have done their work well and the volume is certain to stimulate thought, whether the reader is in accord with its theories or not.—GEO. D. FULLER.

Methods of analysis

A revision of the *Official and tentative methods of analysis of the association of official agricultural chemists*⁷ is ready for distribution. This standard work will be needed by every physiological chemist and analyst. It is published as a second edition, although the volume issued in 1921 was also called a second edition. The revision brings the results of five years of improvement of methods (1919-1923) into more accessible form. The method of cross reference has been changed to give the page, rather than the chapter and section number. Two chapters are added, one on liming materials, and the other on gelatin. There are some changes in arrangement of chapters, Soils now appearing immediately after Fertilizers; and the chapters on Foods and Feeding Stuffs, and Saccharine Products, are now merged in a chapter on Sugars and Sugar Products. Other minor changes will be noted. There are over 100 additional pages in the revised volume. It is a most useful compendium; and fills a need for authoritative information on the best methods for technical analysis.—C. A. SHULL.

⁷ Official and tentative methods of analysis of the Association of Official Agricultural Chemists. 8vo. pp. xvi+535. Published by the Association, Washington, D.C. 1925.

THE BOTANICAL GAZETTE

October 1926

ORGANIZATION AND SIGNIFICANCE OF LENTICELS IN DICOTYLEDONS

II. LENTICELS IN RELATION TO DIFFUSE STORAGE RAYS OF WOODY STEMS¹

RALPH H. WETMORE

(WITH PLATES VII-X)

In a previous article, cauline lenticels were shown to be related definitely to storage rays of the aggregate and compound type. Moreover, the type, size, and distribution of the lenticels on the stems of these forms were shown to reflect the internal organization of the radial storage structures within. In contrast with the relatively few species with aggregate or compound storage organs, the greater proportion of our trees and shrubs possess diffuse storage rays, although the diffusions are of extremely varying extent. It is more general for the original storage aggregations to be broken up into multiseriate units of one to four cells in width. Representative of this are fig. 2, of *Prunus pennsylvanica*, and fig. 10, of *Cephalanthus occidentalis*. In contrast with these, other forms do not have the diffusion carried to such an extreme degree, and the multiseriate entities are 5-12 cells wide. Such a condition is apparent in *Fagus grandifolia* (fig. 14) and *Kalmia latifolia* (fig. 17). Of course all transitions occur between these two groups; there is clearly no line of demarcation. Since the forms of diffuse rays are so numerous, however, the writer has taken this arbitrary method of grouping, in an endeavor to show that the nature of the lenticular structures is corre-

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

lated intimately with the character of the ray. No idea of permanent classification or grouping is intended thereby, the division being but a matter of convenience. In an equally arbitrary manner, the writer has divided the first group again into those in which the rays remain the same size in the phloem as in the xylem, as was shown in the photographs of transverse sections of *Prunus pennsylvanica* (fig. 2) and *Cephalanthus occidentalis* (fig. 10), and those in which the multiseriate rays tend to become V-shape in the phloem region, as is apparent from fig. 27, *Tilia americana*, and fig. 30, of *Carya ovata*.

Type I. Diffuse rays 1-4 cells wide in both xylem and phloem

In these forms, with diffusions of one to four cells in width in both xylem and phloem, we find representatives with transverse and others with longitudinal types of lenticels, as in previous groups. The species of *Betula* and most species of *Prunus* are exceedingly good examples of the transversely oriented structures. Fig. 2 is a photomicrograph of a transverse section of *Prunus pennsylvanica*. Here the horizontally elongate lenticel is distinctly confronting many rays. In this form the pericycle is practically continuous, although occasional thin places and even breaks are found to exist over the rays. Due to the low magnification necessary to include the whole lenticel in the field, this detail is unavoidably indistinct. An interesting condition is apparent in the phloem. The rays seem flanked on one or both sides by lacunae which extend almost down to the cambium. From the point of view of aeration this would be especially valuable. Such a feature is not uncommon and is especially characteristic of all species studied in this genus. The ray cells seem to grow so much larger than the adjacent phloem cells that they are torn away from the latter on sometimes one, sometimes both flanks. The different size of the ray cells in xylem and phloem is apparent, even with this magnification. In the xylem the cells are so small comparatively that no cell structure is visible at all. On the other hand, the cells of the phloem ray are clearly visible.

A study of such sections raises again the question of an anatomical explanation for the transverse orientation of the lenticels. Remembering that in the conifers it was correlated with short rays,

it seemed advisable to note the situation here. Fig. 6 is a tangential view of the wood of *Betula alba* var. *papyrifera*, taken from near the cambium. The multiseriate rays are clearly short, measuring between 0.15 and 0.5 mm. Similarly a tangential view of *Prunus pennsylvanica* (fig. 5) shows an analogous condition in the radial structures. They are slightly wider, averaging three or four cells, but in height they vary between 0.4 and 0.7 mm.

To contrast these conditions with those of stems possessing longitudinal lenticels, the situation in *Prunus virginiana* was studied. It is interesting that *Prunus*, like many other genera, possesses both kinds of lenticels. The majority of the species are as in *P. Cerasus* (fig. 1) and *P. pennsylvanica*, but *P. Padus*, *P. virginiana*, and *P. melanocarpa* show lenticels of longitudinal nature. Any contrasting features between *P. pennsylvanica* and *P. virginiana* should therefore be especially instructive. Figs. 4 and 8 represent transverse and longitudinal sections respectively of this species. Here the single lenticel shown in the first photomicrograph is distinctly subtending a single multiseriate ray. Such rays, as in *P. pennsylvanica*, are flanked by lacunae, a result again apparently of unequal growth of ray and phloem cells. Also, although not visible at the magnification of the photograph, the pericycle is discontinuous and sclerotic, with the interruptions under the lenticels. In tangential section, taken under the same magnification as that of *P. pennsylvanica*, the larger rays appear distinctly longer, measurements revealing them up to 1.5 mm. in length.

In this connection *Cephalanthus occidentalis* (Rubiaceae) is worthy of note. The lenticular conditions are visible in the segment of the stem pictured in fig. 9. Here the large lenticels, especially numerous below the nodes, are clearly longitudinal. Fig. 10 indicates the conditions in the transverse section, and fig. 12 in the tangential view. The former shows that the lenticel is not related to one of these rays, but to many, since the rays are very small and numerous in their diffusion, being but one and occasionally two cells wide. The longitudinal section provides a key to the nature of the lenticel. These rays, although narrow, are not short. They run, in some cases, the whole length of the section; in general, however, they average 2.5-3.5 mm. Moreover, a very much disorganized pericycle is ap-

parent here. The cells can be seen in small groups of two or three each. This condition must facilitate aeration to a very considerable degree.

Many more examples, equally illustrative, exist of this principle. In every case examined, the crucial factor seemed to be the vertical height of the diffuse rays. If the rays are comparatively short, the lenticels are transverse; if longer, they are longitudinal. In general, whatever the condition of the pericycle might be in transverse section, whether continuous or slightly broken, it was usually more disorganized in forms characterized by the longitudinal lenticel. Many other instances of genera like *Prunus* might be given, where both types of lenticels exist, such cases lending themselves exceedingly well to a study of comparative conditions. For example, *Ilex*, *Rhus*, and *Sorbus* serve well this purpose. *Ilex verticillata* has excellent transverse lenticels; *Ilex glabra* possesses longitudinal lenticels. In the former the multiseriata rays average 0.2-0.4 mm. long, whereas in the latter they have a mean extension of 0.7-1.5 mm. In *Rhus*, *R. Vernix* has transverse lenticels, and the diffuse rays in no case measured more than 0.6 mm. in height. On the other hand, *R. typhina* and *R. toxicodendron* have longitudinal lenticels, the larger rays in the former averaging about 1 mm. in height, and in the latter often reaching a length of 1.2 mm. In *Sorbus* a comparable situation exists. *S. domestica* has horizontally elongated lenticels, with multiseriata rays measuring 0.1-0.35 mm. in vertical extension; *S. americana* and *S. intermedia* show small longitudinal lenticels, while the rays in these two species are longer, running up to 0.6 mm.

Many more isolated examples can be given. Thus *Ligustrum vulgare* has transverse lenticels and rays averaging about 0.5 mm. in height. On the other hand, *Rhamnus cathartica*, *Pyrus melanocarpa*, *Maackia amurensis* var. *Buergeri* all have longitudinal lenticels, and rays averaging 0.5-1.0 mm. in height. These examples lead to no absolute results, in the sense that below a certain height of ray the lenticels are transverse, and above that height they are longitudinal. Certainly this is not the case. In fact, the evidence might be considered conflicting except when considered in the light of genera where the two types of lenticels coexist. Here the comparative

status of the problem becomes apparent. One cannot examine numerous sections of many of these species, of which only a representative number have been referred to here, and not be impressed with the fact that the relationship is a real one. In these genera those forms which in the diffusion of their foliar rays have gone farthest, producing bands of tissue but few cells high and few wide, are best aerated by transverse lenticels which subtend many of these comparatively short rays. Where the diffusion of the rays results in multiseriate entities of longer dimension, the longitudinal lenticel will obviously more efficiently aerate the structure. If the rays are very narrow, indicating that the diffusion has gone very far in this direction, the lenticels may be broad as well as long, as for example *Cephalanthus occidentalis*, and thus many may be aerated by one lenticular structure. In such a case the lenticels are usually fewer in number. On the contrary, if they are small, each confronting a single ray, they are invariably more numerous. This relation between the size of the lenticels and their number was shown by DEVAUX (1). Isolated species studied are of little value in solving the problem, for there are no others to compare them with definitely; but once a conclusion is reached from the genera permitting an actual contrast of conditions, because of both types being present, the relationships in others can be understood. If one examines the lenticels and finds them transverse, the storage rays will be found relatively short. Conversely, if the lenticels are longitudinal, these rays will be comparatively longer. In no case did the writer find outstanding exceptions to this generalization. Moreover, a secondary factor was found in the sclerotic pericycle. It is rather an exception than a rule to find a continuous pericycle with longitudinal lenticels. Usually, if not disorganized into nests of cells of varying numbers, it is at least interrupted under the lenticel itself. Thus in general again, the lenticular device seems that best correlated with the internal anatomy to provide gas exchange efficiently to the particular type of ray in each individual case. Although uniseriate rays are present, their part is seemingly, as STRASBURGER (4) claimed, but an adjuster of sap flow throughout the stem. At no time did the writer find intercellular spaces in these structures.

**Type II. Diffuse rays more than four cells wide in both
xylem and phloem**

In the stems possessing larger diffusions, internal conditions in general are much as in those of the preceding type. However, two additional features worthy of note early became apparent. The first of these is the scarcity of forms possessing transverse lenticels. In all the species examined, but two of these were located (*Ribes alpinum* and *R. multiflorum*). All others, and there were many, bore longitudinal aerating structures.

The second feature was the scarcity of arboreal representatives having this type of diffuse ray. All but three (*Fagus grandifolia*, *Oxydendrum arboreum*, and *Parthenocissus tricuspidata*) were shrubs. Parallel to this situation was another, that all of these fruticose forms with wider rays were discovered to have the initial periderm deep seated; that is, it originated within the pericycle, in immediate contact with the phloem. In this way epidermis, cortex, and pericycle were early lost, and the lenticels existing in this periderm were therefore in immediate contact with the stele. STAHL (3) first recognized this last fact, but treated it as of no immediate advantage, except that it afforded him definite proof that lenticels might arise directly from phellogen as well as under stomata. He referred to this situation as being a very common one, and listed as examples, among others, *Berberis*, many species of *Spiraea*, and various species of *Ribes*. DEVAUX (1) also mentioned these deep seated lenticels. The fact that this situation is so characteristic of shrubs seems one worthy of note, and especially so since it is primarily a characteristic of only those shrubs possessing wider diffuse rays. Not a single example was found that showed a periderm of deep origin, and at the same time diffuse rays but a few cells wide. In this connection it is instructive to know that very few of the trees examined possessed the wider type of diffuse ray. Naturally, wider sheets of parenchymatous tissue would be disadvantageous from a mechanical point of view in arboreal forms. Moreover, in their rhytidome and secondary bark formation, trees possess the equivalent of the deep seated periderm of shrubs. In contrast with the larger forms, shrubs can possess wider rays without serious mechanical disadvantage. With their smaller, slow growing stems and branches they can

lay down an initial periderm within the pericycle, and thus complete at once what the tree accomplishes ultimately only through a series of secondary periderm formations.

Considered from the point of view of aeration, deep seated lenticels become very significant. Primitively the lenticels were separated from the central cylinder by both cortex and pericycle. It is true that these possessed intercellular spaces, a generally recognized fact, making aeration possible, but the absence of pericycle and cortex would certainly facilitate gas exchange very materially. This is apparent in the tendency toward a discontinuity in the pericycle, especially under the lenticels of the longitudinal type. The loss of both pericycle and cortex seems a logical step in nature's endeavor to increase this vital activity in accord with the needs of the plant.

As illustrating the situation in the two arboreal forms which have larger diffuse rays, it will be well to refer to *Fagus grandifolia*. It is worthy of note that this form possesses a superficial periderm. Fig. 11 indicates an external view of a portion of a branch of this species. The lenticels here are longitudinal, small and scattered. Fig. 14 portrays a transverse view, showing the large diffuse rays, which average 8-10 cells in width. Opposite one of these is a lenticel. Such was invariably the situation in this species, lenticels always subtending these larger rays. It is interesting that STAHL (3) refers to a similar situation in *Platanus*, although no importance was attached to the fact. In *F. grandifolia*, it should be mentioned also that interruptions in the otherwise continuous pericycle are commonly found under the lenticels. *Oxydendrum arboreum* is an arboreal ericaceous form and is also deserving of attention, since it is the only member of this family that the writer found to possess superficial periderm. The rays here are long vertically, and are frequently arranged in longitudinal rows, so that one can understand the presence of longitudinal lenticels.

As stated, by far the greater number of species of type II have a deep seated periderm. The only two forms of this group with transverse lenticels disclosed by these investigations are *Ribes alpinum* and *R. multiflorum*. Fig. 13 indicates the external condition of *R. alpinum*. The transverse lenticels are very prominent and extend a considerable portion of the way around the stem. Internally, it is

very significant that the multiseriate rays are short, even though they are much wider than usual (fig. 15). In height they average 0.6-0.8 mm. This correlation of orientation of lenticel and height of ray is again remarkably in accord with the other results of this research. *R. multiflorum* proves a similar case.

In contrast with these two forms with transverse lenticels are those possessing longitudinal lenticels. Here, as has been mentioned, belong a great many of our ordinary shrubs. All fruticose members of the Ericaceae examined (twenty-one species in all) were of this type. Here also are included the ten species of *Spiraea* examined. *Berberis vulgaris* and *Lonicera ovalis* are further common examples as well, and many others might be listed.

Kalmia latifolia illustrates conditions in the Ericaceae. Fig. 16 shows a portion of the stem, and fig. 17 a cross-section of the same. The former clearly shows the long vertical lenticels, resembling grooves. The latter equally as clearly connects these lenticular grooves with the ends of the multiseriate rays. The illustration permits one to see that the cortex and pericycle are gone, and that the periderm of alternating layers of cork and phelloid is continuous except at the lenticels. These are indented, as are the annual rings in the region of the rays, and by reason of the minimal amount of complementary tissue present, they might not be considered as lenticels. Their location and correlation, however, show them undoubtedly to be lenticular in nature. Tangential sections prove that the larger rays, occurring under the lenticels, are of considerable depth vertically. In view of the length of the lenticels themselves, this situation is significant.

In the Ericaceae there were sectioned and studied thirteen species of shrubs. In every one of these the same situation is found, except that the lenticels usually are not so elongated vertically. In all cases the secondary longitudinal lenticels are related to multiseriate rays of varying width, which often reach a height to be measured in millimeters, and not in fractions of a single millimeter. Another feature is characteristic of the greater number of these forms. On reaching the phloem the rays immediately broaden so that the outer ends of these structures confronting the lenticels are many times wider than the inner ends in contact with the xylem. In this way a greater surface of the ray is exposed, thus facilitating aeration.

The situation in the various species of *Spiraea* is especially instructive. The modes of distributions of the lenticels are striking, as they differ to such an extent in the different species. But three examples will be used here as illustrative of the group. Fig. 18 indicates the situation in *S. Veitchii*. A nodal or leaf scar is visible near the top of the section. Below this on either side a definite row of lenticels can be seen, these rows continuing downward for a considerable distance. A few small lenticels can be detected elsewhere on the stem. This general situation is true for every node, after the cortex and pericycle have been exfoliated through the periderm formation. In fig. 21 can be seen three illustrations of *S. gemmata*. Here the lenticels are few in number and grouped around or near the node, occasionally running down directly below it, in one median cluster, for a short distance. Fig. 19 represents a short portion of *S. salicifolia*. Here a single row of lenticels is found above the node, while below this point there are two flanking lines of these structures extending downward for a considerable distance. Other species with their characteristic lenticular arrangements might be indicated as well, but these will serve as types. The clue to the problem of these variable distributions is furnished by the tangential sections. Fig. 22 is such a view of *S. Veitchii*. Below the leaf trace the ray quickly becomes diffuse, breaking up into multiseriations of varying widths. The interesting part is that these multiseriate units are uniformly distributed, except on either side below and lateral to the trace, where a row of these rays is found running vertically in the stem. The larger size of the components of these rows makes them very distinct. Could more of the tangential section have been shown, they would have been found to extend a considerable distance downward, until in proximity to another trace. The situation in *S. salicifolia* is very similar below the node, the lateral rows of multiseriate rays being very pronounced (fig. 23). Extending above the node can be seen a part of the undivided portion of the ray. The flanking lenticels below the trace and the single row of lenticels above it thus seem to have distinct internal relationships. A similarly oriented section of *S. gemmata* is shown in fig. 24. Here it is evident that the grouped rays separate quickly, and a short distance below the node no lateral rows are apparent; in fact, the groupings so quickly become diffuse that the storage aggregation soon cannot be differenti-

ated from those in the remainder of the field. In this form it will be remembered that a cluster of lenticels is located around the node, being apparent below this point for only a very short distance. In other species of *Spiraea* the lenticels are as uniformly scattered just below the leaf scars as elsewhere. In these cases scarcely a sign of a concentration of multiseriations near the node is apparent in tangential section, so quickly do they become diffuse.

An epitome of this situation seems to be found again in the correlation of ray and lenticel. This is very strikingly illustrated in *Spiraea* and the Ericaceae. Whatever specific type of diffusion exists in the storage ray, it is closely paralleled by the superficial distribution of lenticels. Still further is the aeration facilitated by the early loss of epidermis, cortex, and pericycle. The fact that the multiseriate structures frequently lie in vertical lines also explains the equally frequent corresponding vertical rows of lenticels. The concentration of lenticels below the nodes, as in *Spiraea*, has been noticed by earlier botanists. TRÉCUL (5) referred to the occurrence of a half-crown of lenticels below the leaf insertion in *Ficus Carica* and some willows. DEVAUX (1) mentions that the only lenticels found in *Daphne* and *Veronica* are localized in places in the neighborhood of the leaf scar; and other forms, like *Ailanthus glandulosa*, *Sambucus nigra*, *Aralia Sieboldii* (*Fatsia japonica*), etc., have them more conspicuously in the nodal region than elsewhere. When they do exist, he claims, they exist in pairs, one on either side of the axillary bud. Certain herbaceous forms have a number of lenticels on the actual leaf scar, as, for example, *Malva rotundifolia*, *Melilotus macrorhiza* (*M. officinalis*), *Brassica nigra*, etc. In summary, he states: "Il existe donc aux noeuds spécialement au-dessous de la feuille, des conditions internes persistantes, qui ont dès le début une grande influence sur le développement lenticellaire, quelle que soit l'origine des lenticelles." Since he was explaining lenticels in terms of transpiration, "des conditions internes persistantes" completely evaded him. However, specific instances of relationship between lenticels and rays have been noticed in the past. Thus SCHENCK (2) mentioned that the larger rays of *Aeschynomene sensitiva* were in direct connection with the lenticels, and were so aerated. STRASBURGER (4), although he quoted SCHENCK, gives no specific cases, but he does say

in general that the rays are aerated directly from the lenticels through the aeriferous cortex. It seems from the many forms studied by the writer that any such relationship of the lenticels to the nodes is due to their proximity to storage rays within. Whether there is one on either side, as DEVAUX reports in *Daphne* and *Veronica*, and as the writer has commonly found in *Berberis vulgaris*, *Parthenocissus tricuspidata*, etc., or whether a circle exists below, as TRÉCUL (5) noted in *Ficus Carica*, or whether varied conditions exist as in *Cephalanthus occidentalis* or *Spiraea*, there is seemingly a direct correlation with the type of ray within. STAHL (3) mentions *Abies pectinata* as having nodal lenticels. The situation here, however, is very different.² The nodal relationship in the conifers is a primitive one, due essentially to the presence of an aerating device, the parichnos, in at least the more primitive forms. In the angiosperms the grouping near the node is of different significance. The lenticels are now related to the storage elaborations instead of to the traces themselves as in the conifers. There is also the tendency in these forms with wider diffusion for the multiseriate structures to be longer vertically, either as a result of the individual rays not being broken up into shorter units, or else by the retention of these in longitudinal rows. In these higher forms, therefore, the lenticels accordingly are often very preponderantly extended vertically. Thus, one might say that in the evolution of the lenticel, the transverse type is being replaced by the longitudinal in these forms, for thereby the physiological need of gas exchange is much more readily supplied. Moreover, not only single longitudinal lenticels, but often vertical rows of these are present, thus insuring the aeration of the correspondingly situated rays within.

Type III. Diffuse rays 1-4 cells wide, tending to become V-shaped in phloem

As stated earlier, the diffuse rays present in angiospermous trees are almost invariably only a few cells wide. These rays of narrow dimensions, however, usually tend to become very much wider in the phloem, until they are pronouncedly V-shaped. In this way the

² JEFFREY, E. C., and WETMORE, R. H., Ann. Botany. Unpublished.

rigidity of the tree is not lessened to any extent, while the provision for increased aeration is magnified many times.

It is interesting that certain shrubs possess indications of this situation. Thus it was mentioned that practically all fruticose Ericaceae studied showed a tendency for the rays to become wider in the phloem, especially in close proximity to a lenticel. *Myrica asplenifolia* offers an instance of the same thing happening under lenticels only; however, the situation is not common nor characteristic in shrubs. It is only in arboreal forms that we can see a complete series indicating how this so-called V-shaped phloem ray has its origin, and to what degree it has evolved in some forms.

Salix provides a noteworthy primitive condition in these phloem rays. Fig. 25 shows a transverse section of *Salix alba* var. *vitellina*. Here the ray under the lenticel is clearly V-shaped, although not starting to fan out until some distance from the cambium. On either side can be seen a single instance of the same phenomenon, but to a less marked extent. In the xylem all diffuse rays look alike, but all of these do not become V-shaped in the phloem. The pericycle opposite those which do becomes discontinuous, and is found only over the phloem groups, although this is not apparent at the magnification of the photograph.

The situation in *Carpinus cordata* is equally instructive. It is interesting to note that in this genus certain species have aggregate storage rays (*C. caroliniana* and *C. japonica*), while *C. cordata* possesses the diffuse type. In this species were observed (fig. 20) the largest lenticels discovered. A transverse section (fig. 26) shows the internal situation. The lenticels are remarkably broad for those of longitudinal nature, covering a large number of diffuse rays. However, the figure shows that but a small number of the latter become V-shaped. It is worthy of note that the pericycle becomes disorganized opposite these rays, bringing about a close connection between the lenticel and the V-shaped rays. That this pericycle is continuous elsewhere should be mentioned. These phloem rays become of considerable length and clearly abut on one another when seen in tangential view, so that the large lenticels obviously constitute an advantageous correlation. It may be pointed out, however, that smaller lenticels in greater number would subserve the same purpose as well. This will be apparent in the following examples.

Benzoin aestivale (fig. 3) is definitely of a higher type. Here the larger rays all tend to become V-shaped in the phloem, due to meristematic activities in the rays themselves. The phloem masses alternating with these rays are all clearly subtended by sclerotic nests of the discontinuous pericycle, but none of these cell groups appear opposite the rays. Not all of the rays have lenticels confronting them in this particular section, but serial sections indicate that such an organ exists for practically every one. Fig. 7 illustrates the great number of these small lenticels, and the tendency for them to become arranged in rows. The multiseriate rays in the xylem vary between 0.2 and 1.1 mm. in height, but in the phloem they average about 2 mm. This increase in length of rays in the phloem is a character frequently found, but especially so with those which become V-shaped. It is this situation that enables one to explain the vertical rows of lenticels.

In *Tilia americana* (fig. 27) the cross-section shows practically every multiseriate ray with the tendency to become V-shaped, although in varying degrees. This is brought about by definite anticlinal divisions in the cells of the rays, as is apparent from the tangential view in fig. 28. Here the cells of the rays are clearly in transverse periclinal rows, which would be possible only by divisions in an anticlinal direction. One is not able to identify any definite meristem, however, any and all cells seemingly being capable of division. That this is true is easily discernible under greater magnification, for cells of all sizes are apparent, some being recent and some older products of division. This tangential section distinctly indicates the great length of the rays in the phloem. In the xylem the longest ray measured was 2 mm., while in the phloem they varied between 3 and 4 mm. In accord with this fact, there is present again a tendency for the lenticels to be distributed in vertical rows of two or three, as shown in fig. 31, a superficial view of a portion of a branch of *T. americana*.

Of a nature still more striking, although similar in several respects, is the situation in *Carya ovata*. Fig. 30 is a transverse section showing the large V-shaped rays, and the intervening pointed phloem masses capped with nests of pericycle cells. The lenticels in this species are very small, but over each of the three phloem V's shown is a lenticel, indistinctly visible. Extending between the

bottom of each of these V's and the lenticular organ, there is distinctly apparent a light area, visible in both ray and cortex. The situation here noticeable is much more distinct in fig. 32. This represents a portion of one of the V-shaped rays in tangential view, under higher magnification. There is evident from top to bottom a definite meristem, the cells on either side being remarkably seriate. The light appearing areas visible in transverse section, therefore, are regions of cambial activity. That the cortex should assume these divisions as well is of additional interest. The height of the rays in *Carya ovata* offers an interesting study. In the xylem no multiseriate ray was found which measured over 1.3 mm. In the mid-phloem region, a maximum length of 6 mm. exists for these rays. In striking contrast with this, just within the pericycle the rays measure 9-14 mm. In other words, the rays increase from seven to ten times in length in the phloem. This is truly remarkable, and must indicate transverse anticlinal divisions as well as vertical. The lenticular situation accompanying these internal highly evolved structures is very instructive. Fig. 29 is a portion of a branch of *Carya ovata*. The vertical rows of small lenticels are undoubtedly like those seen in the transverse section. Moreover, removal of the bark shows that they are directly over the large rays. The lenticels in these rows are so approximated that they almost constitute a single very long structure. The fissures from lenticel to lenticel are practically continuous. It is not surprising, therefore, that the identity of these small lenticels is entirely lost in the elongated structures of *Amelanchier oligocarpa* (fig. 33). This type of lenticular relationship with V-shaped phloem rays is comparatively common in trees of various affinities, as *Carya glabra*, *Castanea dentata*, *Liriodendron Tulipifera*, *Fraxinus nigra*, etc.

In summary, the V-shaped ray in the phloem is a structure which originates through active cell division of the initial multiseriate ray. Although usually these divisions are diffuse, they may in certain forms become so localized as to form a definite cambium extending the entire radial length of the ray, and even across the cortex to the lenticel. Also, as a result of other divisions, the ray increases in length as well as in width, until a very broad front is in juxtaposition to the lenticel. As a result of these meristematic activities, the peri-

cycle becomes broken up into nests which are localized at the peaks of the phloem masses. The lenticels are all longitudinal, are invariably opposite the phloem V's, and in general are arranged in vertical rows, limited more or less in length by the extension of the ray within. Although occasionally large, they are usually small and numerous, and frequently so close together that with age the individual limits are lost and the structure assumes the appearance of a single monstrous complex.

The advantage in the evolutionary development of this type of structure in trees is not difficult to picture. In order to give the maximum rigidity to these arboreal forms, so that they might successfully withstand the winds, the uniformly diffuse storage ray of small dimensions has evolved. In this way the stele is interrupted to a minimal amount in any one place by living parenchymatous tissue. Then to supply the maximum storage so necessary to the efficiency of these angiospermous forms, the V-shaped phloem ray has evolved. Here it in no way impairs the mechanical rigidity of the stem in relation to violent stresses and strains. Moreover, it has an additional advantage in that the major part of the storage tissue is placed near the periphery of the stem, thereby greatly increasing its chances for aeration. Originally it seems that only those rays nearest the lenticels assumed meristematic activities and became V-shaped. In higher forms like *Tilia americana*, *Carya ovata*, etc., practically every multi-seriate ray assumes this form in the phloem, and associate vertical lines of lenticels are found to exist with each. As a consequence, one cannot help but think of the evolution of storage rays in the phloem of the cylinder as closely correlated, to a great degree at least, with the function of aeration. This is borne out in a most striking manner by the external evidence supplied by the arrangement of the superficial aerating organs, the lenticels. The funnel-like nature of the rays in the phloem facilitates the aeration of the living elements of the stele.

The only reference which the writer has found to these V-shaped rays is that of STRASBURGER (4). He mentions these structures as possessing intercellular spaces in *Tilia*. This condition has been confirmed time and again in many species by the writer. The literature seems to furnish no reference to the generally diffuse meristematic

development in the forms discussed here, nor of the presence of an actual cambium in *Carya*.

Discussion

The study of forms possessing the three types of diffuse rays, as artificially divided in this research, results in at least one very evident conclusion. There seems to be no doubt of a very close correspondence between the elements of the foliar ray and the lenticular structure evolved for its aeration. This correlation is so apparent that a superficial study of the lenticular devices enables one to predict with considerable accuracy the character of the rays which they cover; and vice versa, study of the transverse and longitudinal sections permits one equally well to be reasonably certain of the type and distribution of the lenticular organs which subtend them.

It is of interest to note that forms with transverse lenticels are decidedly in the minority among those species possessing diffuse storage rays. Generally only those more primitive trees and shrubs which have retained the narrow, short, diffuse rays, without modification, possess these structures. Usually, wider multiseriations are at once correlated with the longitudinal lenticel. Thus we find *Spiraea salicifolia* and others possessing lines of longitudinal lenticels confronting lines of vertically elongated, large, multiseriate rays; and in *Kalmia latifolia*, single, greatly extended longitudinal lenticels corresponding superficially to each immensely long ray. Still further is this type of lenticel advantageous when the rays in the phloem acquire meristematic activities and increase not only their length, but their transverse dimension as well, as exemplified in the V-shaped rays of this part of the stele. Here again not only single longitudinal lenticels, but rows of them appear, as in *Carya ovata*, *Amelanchier oligocarpa*, etc. It is thus fitting to conclude that the longitudinal lenticel represents the greatest evolutionary advance, and naturally, therefore, the internal structures accompanying present the greatest range of variability.

Summary

1. Lenticels occurring on shrubs and trees possessing diffuse storage rays are directly related in position, structure, and size to those

rays, just as they were in cases of forms with aggregate or compound rays. In this way the aeration of the rays and other living tissues is affected.

2. Transverse lenticels exist much less frequently on forms with diffuse storage rays than do longitudinal lenticels. When present, they are universally associated with vertically shorter storage rays.

3. Longitudinal lenticels are more characteristic of woody forms with diffuse storage rays. In these cases the rays are always longer vertically. Frequently they are very long or they are arranged in vertical rows. At such times the rays are confronted by greatly elongated lenticels or vertical rows of lenticels.

4. Some trees, as *Tilia*, *Carya*, *Fraxinus*, possess V-shaped rays in the phloem. These rays become greatly elongated as well. Such increases in width and length are brought about by meristematic activities in the phloem region of the ray. These broad rays are confronted by rows of lenticels, thus facilitating their aeration.

5. The general existence of a deep seated periderm in shrubs and of a rhytidome in trees makes possible a still more intimate connection between the lenticels and the rays within.

6. The organization and evolution of the storage rays in the stems of angiosperms has been accompanied by a parallel organization and evolution of the lenticels. In the root, however, the more primitive transverse lenticels in their original relationship to the appendages have been retained. This is in accord with the conservative nature of the root.

In conclusion, the writer wishes to express his appreciation of the criticism and advice of Professor E. C. JEFFREY during the course of this research.

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EXPLANATION OF PLATES VII-X

PLATE VII

FIG. 1.—Portion of trunk of *Prunus cerasus* with horizontally elongated, transverse lenticels; $\times \frac{1}{8}$.

FIG. 2.—Portion of transverse section of *Prunus pennsylvanica*, showing relation of transverse lenticel to several multiseriate rays; $\times 25$.

FIG. 3.—Portion of transverse section of *Benzoin aestivale*, with V-shaped rays in phloem; $\times 25$.

FIG. 4.—Portion of transverse section of *Prunus virginiana*, showing relation of longitudinal lenticel to single multiseriate ray; $\times 25$.

FIG. 5.—Tangential section of *Prunus pennsylvanica*, showing height of multiseriate rays; $\times 40$.

FIG. 6.—Same for *Betula alba* var. *papyrifera*; $\times 40$.

FIG. 7.—Portion of stem of *Benzoin aestivale*, showing lenticels; $\times 1$.

FIG. 8.—Tangential section of *Prunus virginiana*, showing height of multiseriate rays; $\times 40$.

PLATE VIII

FIG. 9.—Segment of stem of *Cephalanthus occidentalis*, showing lenticels; $\times 1$.

FIG. 10.—Portion of transverse section of *Cephalanthus occidentalis*, showing relation of large longitudinal lenticel to many diffuse rays; $\times 27$.

FIG. 11.—Portion of branch of *Fagus grandifolia*, showing lenticels; $\times 1$.

FIG. 12.—Tangential section of *Cephalanthus occidentalis*, showing great length of rays; $\times 40$.

FIG. 13.—Segment of stem of *Ribes alpinum*, with very prominent transverse lenticels; $\times 1.5$.

FIG. 14.—Portion of transverse section of *Fagus grandifolia*, showing relation of lenticel to a large ray; $\times 27$.

FIG. 15.—Tangential section of *Ribes alpinum*, showing short wide rays; $\times 40$.

FIG. 16.—Segment of stem of *Kalmia latifolia*, with very long longitudinal lenticels; $\times 1.5$.

FIG. 17.—Transverse section of same, showing relation of lenticels to multiseriate rays; $\times 10$.

PLATE IX

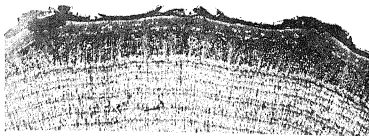
FIG. 18.—Segment of stem of *Spiraea Veitchii*, with lenticels in two flanking lines below node; $\times 1.5$.



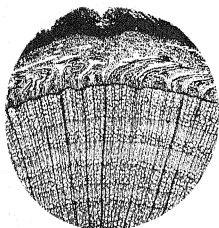
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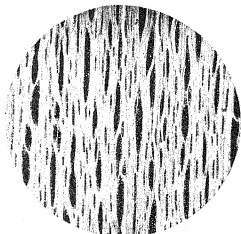
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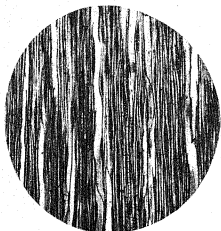
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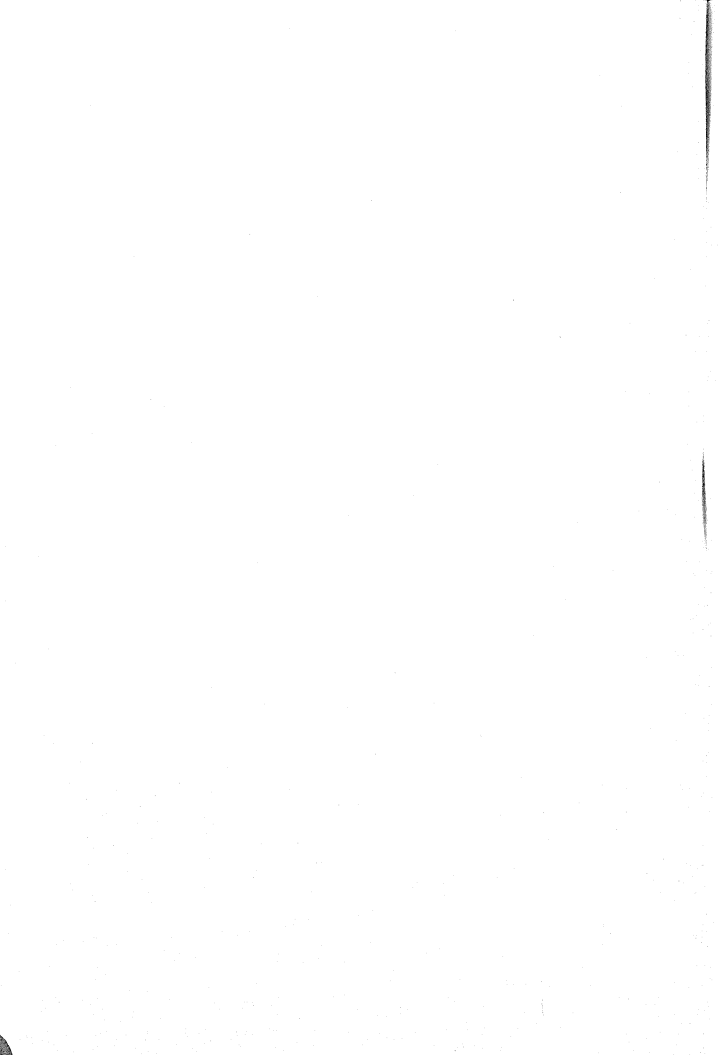
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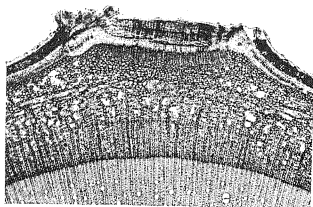


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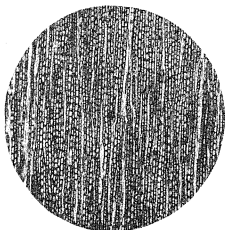
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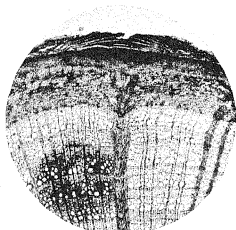
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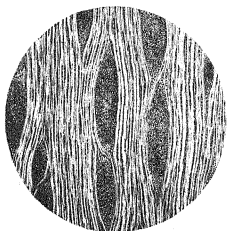
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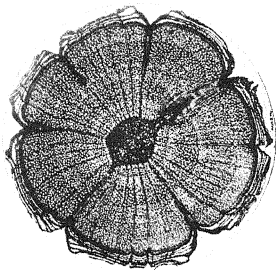
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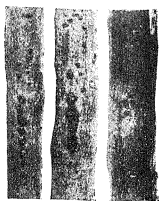
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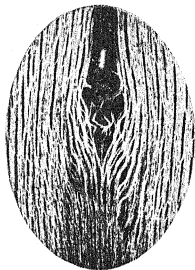
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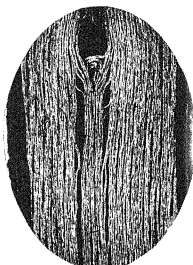
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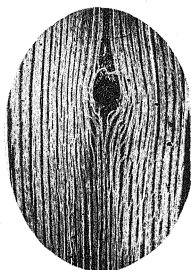
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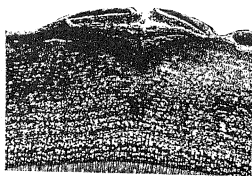
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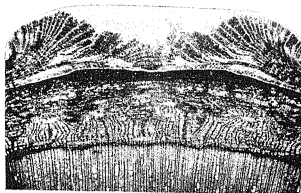
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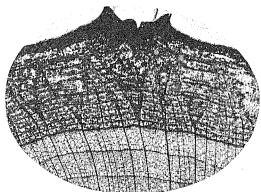


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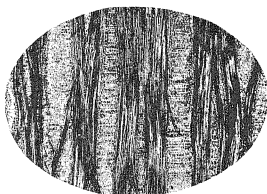


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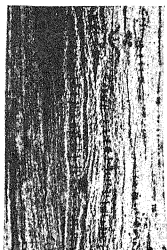




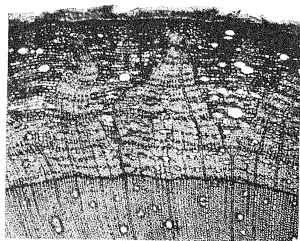
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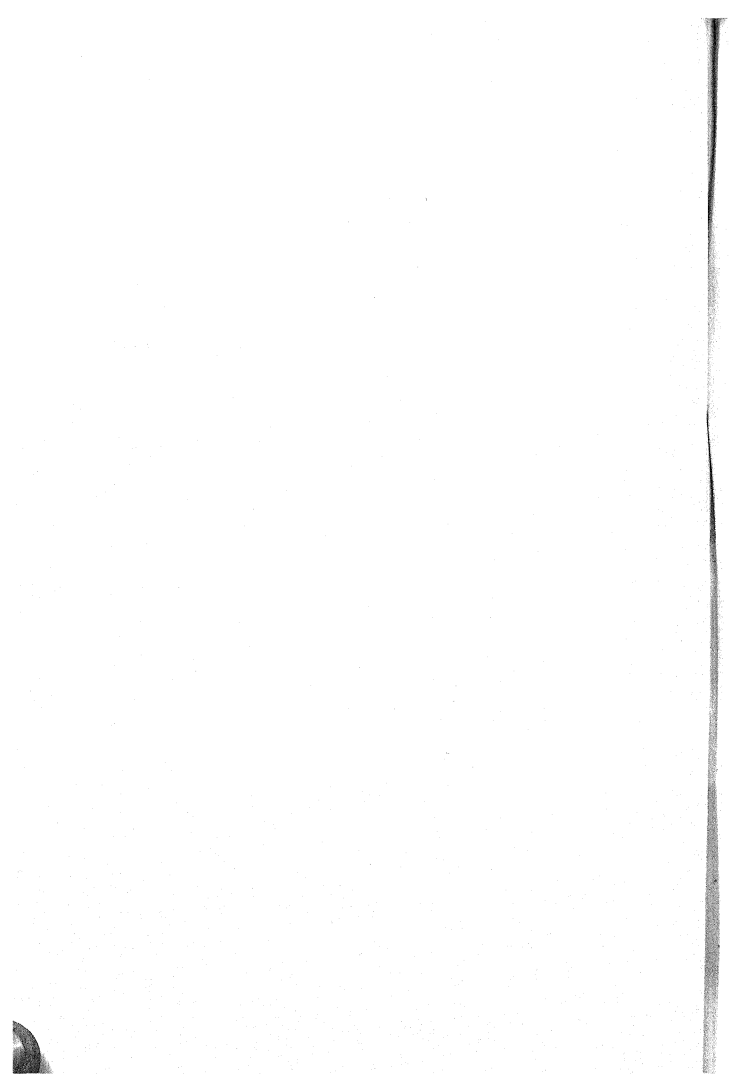


FIG. 19.—Segment of stem of *Spiraea salicifolia*, with lenticels in two flanking lines below node, and undivided line above; $\times 1.5$.

FIG. 20.—Segment of branch of *Carpinus cordata*, showing immense longitudinal lenticels; $\times 1$.

FIG. 21.—Segments of stems of *Spiraea gemmata*, with lenticels clustered at nodes; $\times 1$.

FIG. 22.—Tangential section of *Spiraea Veitchii*, showing two flanking lines of multiseriate rays below node; $\times 10$.

FIG. 23.—Same for *S. salicifolia*; $\times 10$.

FIG. 24.—Same for *S. gemmata*, showing multiseriate rays clustered at node; $\times 10$.

FIG. 25.—Portion of transverse section of *Salix alba* var. *vitellina*, with V-shaped rays in phloem, especially under lenticel; $\times 25$.

FIG. 26.—Portion of transverse section of *Carpinus cordata*, showing lenticel and its relation to V-shaped rays of phloem; $\times 22$.

PLATE X

FIG. 27.—Portion of transverse section of *Tilia americana*, showing V-shaped phloem rays and relation of lenticel to one of them; $\times 25$.

FIG. 28.—Tangential section of phloem of *Tilia americana*, showing V-shaped rays; $\times 40$.

FIG. 29.—Segment of branch of *Carya ovata*, with vertical lines of longitudinal lenticels; $\times 1$.

FIG. 30.—Portion of transverse section of *Carya ovata*, with prominent V-shaped phloem rays, each subtended by small lenticel; $\times 25$.

FIG. 31.—Segment of stem of *Tilia americana*, with short vertical rows of longitudinal lenticels; $\times 1$.

FIG. 32.—Tangential section of single large V-shaped ray of phloem of *Carya ovata*, showing presence of actual cambium; $\times 40$.

FIG. 33.—Segment of branch of *Amelanchier oligocarpa*, with longitudinal lenticels much elongated vertically; $\times 1$.

INFLUENCE OF MINERAL ELEMENTS UPON DEVELOPMENT OF CHLOROPLAST PIG- MENTS OF SOY BEANS*

CARL G. DEUBER

(WITH FIVE FIGURES)

Introduction

Cultural experiments with plants have demonstrated many instances in which deficiencies of various mineral elements have produced, in addition to reduced growth, a disturbance in the development of the chloroplast pigments. The commonest manifestation of such a condition is the yellow or white color of the foliage of plants lacking a proper supply of iron. Most of the other essential elements also influence the development of the chloroplast pigments.

VILLE (24), working with hemp plants, found that the greatest decrease in chlorophyll and carotin was brought about by a deficiency of nitrates. Lesser decreases resulted from deficiencies of potassium, phosphate, and calcium in the order named. LESAGE and SCHIMPER (22) noted that excesses of mineral substances reduced the chlorophyll content of plants. REED and HAAS (18) investigated the effects of several ions upon young citrus and walnut trees. They found that chlorosis of the leaves resulted from high applications of chlorine in the form of sodium chloride, from the application of sodium bicarbonate in solutions deficient in calcium, from high concentrations of potassium, and from very low concentrations of magnesium. GARNER and co-workers (7) described chloroses of tobacco plants caused by deficiencies of potassium, sulphur, and magnesium. MAMELI (14) found that within certain limits the development of the chlorophylls (*a* and *b*) was proportional to the magnesium supplied in the nutrient solution. The mottling of *Coleus* plants was associated with a shortage of nitrates by SCHERTZ (20). BOKORNY (2) observed that the first effect of cultivating green algae in solutions lacking calcium was a decrease in the chlorophyll content.

* Portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Missouri, 1925.

Applications of sulphur-containing fertilizers to sweet corn (6), alfalfa (19), rutabagas, parsnips, and beets (3) resulted in a darker green color of the foliage. McHARGUE (12) reported manganese in small amounts essential for proper chlorophyll development. McLEAN and GILBERT (13) have corrected a chlorosis of spinach by spraying with manganous sulphate solutions.

While there is abundant evidence that various mineral elements influence the formation of the chloroplast pigments, very little has been accomplished in showing in what manner this influence is operative. A mineral element may function as a constituent of the chlorophylls as magnesium; it may act as a catalyst in the formation of specific portions of the pigment, as is suggested by ODDO and POLACCI (16) for iron; or it may not have a direct bearing on the pigments themselves but so influence the general metabolic processes, such as respiration, that the normal production of the chloroplast pigments is more or less impaired.

WILLSTÄTTER (25) has shown conclusively that magnesium is the only mineral constituent of chlorophyll (*a* and *b*). The reason for the connection of this element with chlorophyll development demonstrated by MAMELI, GARNER and co-workers, and by REED and HAAS is therefore clear. Nitrogen is also a constituent of chlorophyll. ODDO and POLACCI suggest that iron acts as a catalyst in the formation of the pyrrole groupings which are the basis of the chlorophyll molecules. These investigators arrived at this hypothesis from the successful substitution of a magnesium salt of pyrrole carbonic acid for iron in the growth of plants. Studies by the writer (4) with a similar compound substituted for iron with several plants gave negative results in all trials. We do not know in what way the other mineral elements are connected with the formation of the chloroplast pigments.

Not only is our knowledge limited as to the way in which the mineral elements are connected with the development of the chloroplast pigments, but we also know very little of the effect of an element upon the development of the individual pigments found in the chloroplast. Yet in view of present theories of photosynthesis (1) which associate all four of the chloroplast pigments with this process such information is important. By comparing the absorption bands

of chlorophylls (*a* and *b*) in living leaves, WLODEK (27) has been able to show that a lack of potassium causes an absolute and relative diminution of chlorophyll *b* and an increase in chlorophyll *a*. The data of MAMELI for maize show that increasing the magnesium supply above a certain point results in a decrease in the quantity of carotinoids, although the chlorophylls were further increased.

The work reported in this paper and elsewhere by the writer was planned to determine the effect of several essential mineral elements on the development of the chloroplast pigments, and to secure information on the method of their action. In the present investigation soy bean plants were grown in water cultures, and the influence of iron, potassium, and sulphur in the development of the chloroplast pigments studied by making quantitative determinations of the chlorophylls and the carotinoids.

Methods

The culture vessels employed were 500 cc. wide-mouth bottles wrapped in black paper and provided with paraffined brown paper tops. Three or four Wilson soy bean seedlings were grown in each culture. Iron wire rings with uprights were fastened to the culture vessels with rubber bands to support the tops of the plants. In the experiments with iron salts these supports were new, so that iron from iron rust was not a factor.

Knop's nutrient solution with the following composition was used: $\text{Ca}(\text{NO}_3)_2$, 0.8 gm.; KNO_3 , 0.2 gm.; KH_2PO_4 , 0.2 gm.; MgSO_4 , 0.2 gm.; distilled water, 1000 cc. Separate stock solutions of the constituent salts were prepared 100 times the concentration used in the cultures, dilution and mixing of the salts being made just before renewing the culture solutions every four or five days. Stock solutions of ferric citrate and ferrous sulphate were prepared fresh at each renewal of the solutions, to prevent hydrolysis of the iron salts. Merck's "reagent" and Baker's "analysed" chemicals were used, and the distilled water was obtained from a tripure still.

In the experiments with potassium, the basal Knop's solution was modified to contain 0, 28, 134, and 238 p.p.m. K. This element was omitted from the first solution by substituting NaH_2PO_4 for KNO_3 and KH_2PO_4 . The second solution contained 28 p.p.m. K in

the form of KH_2PO_4 , and the third with 134 p.p.m. K was the regular Knop's solution. The fourth solution contained 238 p.p.m. K in the forms of KNO_3 , KH_2PO_4 , and KCl .

In the experiment with sulphur, the basal Knop's solution was modified to contain 0, 13, 26, and 46 p.p.m. S. Sulphur was omitted from the first solution by replacing MgSO_4 with MgCl_2 . The second solution with 13 p.p.m. S received but half the MgSO_4 of the Knop's solution, the Mg content being maintained by adding MgCl_2 . Knop's solution contains 26 p.p.m. S, the concentration employed in the third solution. The fourth solution with 46 p.p.m. S received Na_2SO_4 in addition to the MgSO_4 of the Knop's solution. Iron was supplied to the solutions of the potassium and sulphur experiments in the form of ferric citrate at the rate of 0.228 p.p.m. iron.

The reactions of the nutrient solutions were determined at the time of preparing the solutions and after the plants had grown in them four or five days, the colorimetric method of GILLESPIE (8) being employed.

The experiments were conducted on central benches in a well lighted greenhouse having an average temperature of 20° C.

In the first experiments with iron and potassium, the chloroplast pigments were determined by extracting the leaves of the plants which had been air dried in the dark at approximately 18° C. In the later experiments the chloroplast pigments were extracted and estimated immediately upon harvesting the fresh plants. The methods of WILLSTÄTTER, modified as suggested by SCHERTZ² for the extraction and separation of the chloroplast pigments were followed. The procedure consisted in extracting the leaf material with acetone, transferring the acetone extract to ether, and saponifying the chlorophylls in order to separate them from the carotinoids. Carotin and xanthophyll were separated by means of their different solubilities in petroleum ether and methyl alcohol.

The acetone extracts of a series of leaf samples were compared relatively in a Dubosque colorimeter. The ether extracts containing

² The writer wishes to acknowledge his thanks to Dr. F. M. SCHERTZ, United States Bureau of Plant Industry, for suggestions regarding the extraction, separation, and quantitative estimation of the chloroplast pigments, and for a sample of purified chlorophyll (a and b) mixed.

all the chloroplast pigments were treated in the same way. The mixed chlorophylls (*a* and *b*) of a given series of samples were compared relatively and with alcoholic solutions of chlorophylls (*a* and *b*), the purified chlorophyll for these standards being furnished by SCHERTZ. Relative comparisons were made of the combined carotinoid samples and of the carotin and xanthophyll.

Influence of iron

Two experiments were performed on the influence of iron as ferrous sulphate and as ferric citrate, on the growth and on the chloroplast pigments of soy bean plants. In the first experiment Knop's solution containing iron as ferrous sulphate at rates of 0.367, 0.734, 1.468, 2.936, 4.411, 5.872, and 8.822 p.p.m., and iron as ferric citrate at rates of 0.228, 0.456, 0.912, 1.824, 2.736, 3.648, and 5.472 p.p.m. Two cultures of three plants each were used for each concentration of iron. The solutions were renewed every four days. The total quantity of iron supplied per plant in milligrams was 1.5 times the concentration in parts per million. The initial hydrogen-ion concentration varied from P_H 5.65 to 5.8, and the maximum final from P_H 6.15 to 6.7, the greater changes occurring with the larger plants.

After twelve days' growth, chlorosis of the new leaves in both series of iron salts was evident with the first three concentrations of iron and the solution lacking iron. The chlorotic condition was soon followed by a black spotting of the youngest leaves. This condition first appeared in the form of minute yellow areas in the leaf blades; these increased to irregular shaped areas less than 1 mm. in size, and underwent changes in color to light red, red, and finally to reddish brown or black. Young leaves were often peppered over their entire surface with these spots and dropped off prematurely. This condition was most evident in the ferrous sulphate series. At the conclusion of the experiment, after 35 days' growth, chlorosis was evident in the first five solutions of the ferrous sulphate series and in the first four of the ferric citrate series.

The growth of the plants was markedly affected by the concentration or amount of iron supplied.³ Increasing the concentration of

³ It is impossible to differentiate in these experiments between concentration and total amount of an element supplied. In a given series the nutrient solutions were

iron as ferric citrate produced improved growth up to 3.648 p.p.m. (fig. 1). Increasing iron as ferrous sulphate increased growth up to 8.822 p.p.m., the maximum used.

Ferric citrate proved to be a much more efficient source of iron than ferrous sulphate at all concentrations employed. A comparison of the growth curves of fig. 1 makes it possible to estimate the

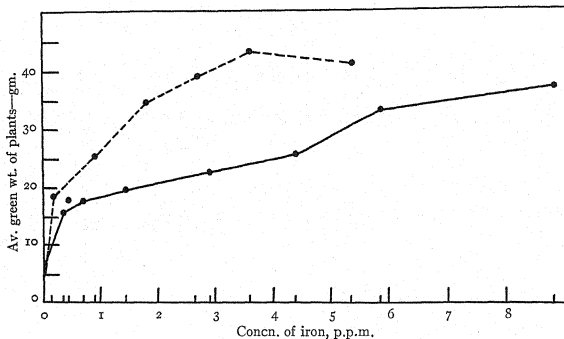


FIG. 1.—Green weights of soy bean plants per culture supplied seven concentrations of iron as ferrous sulphate and ferric citrate (p.p.m. = parts per million). Dotted line, ferric citrate; continuous line, ferrous sulphate.

quantity of iron as ferric citrate which would be required to produce the same amount of growth obtained with the various concentrations of ferrous sulphate iron. Thus to produce 37.155 gm. fresh weight of plants secured with 8.822 p.p.m. iron as ferrous sulphate would require 2.3 p.p.m. ferric citrate iron, or 3.8 times as much iron as ferrous sulphate as in the form of ferric citrate. For a concentration of 5.872 p.p.m. iron as ferrous sulphate this factor is 3.5; for a concentration of 4.411 p.p.m., 4.4; for a concentration of 2.936 p.p.m., 4.5; and for a concentration of 1.468 p.p.m. it is 4.9. In other words,

changed the same number of times. The total quantity of an element supplied varied with the concentration. When the term concentration is used in this paper it is understood that total quantity or amount of the element supplied may be the governing factor and not the concentration.

iron as ferric citrate in the range of concentrations where marked depression of growth did not occur was about four times as efficient in this experiment as iron in the form of ferrous sulphate. Since the highest concentration of ferrous sulphate employed produced no decrease in growth its optimum concentration cannot be stated, but for ferric citrate a concentration of 3.648 p.p.m. iron was the optimum.

Attempts to determine the plastid pigments were made by extracting with acetone two gm. samples of leaves dried as previously described. When the green pigments of the acetone extracts were transferred to ether, some of the greenest leaf samples gave a very green wash water, which would not give up its coloring matter to ether, petroleum ether, or carbon bisulphide. More pigment was transferable to ether from the acetone extracts of some of the chlorotic leaf samples than from that of some of the greenest and most vigorous plants. Evidently, during the drying of the leaves some of the chloroplast pigments were decomposed to a water soluble form. Comparisons of the total pigment material extracted from dried leaves by 80 per cent acetone appeared unreliable. In the ferric citrate series the highest pigment content was found in the plants grown in a solution containing 0.456 p.p.m. iron. These plants had yellow to light green leaves. From these results it was concluded that acetone extracts of dried soy bean leaves are unsafe criteria for the estimation of the chloroplast pigments, and that satisfactory quantitative determinations of the chloroplast pigments may be seriously interfered with by changes in the pigments occurring during the drying of the leaves. Although WILLSTÄTER (11) used the dried powder of leaves for all ordinary extractions, he found that drying caused a loss of chlorophyll, and when the leaves were not properly dried the pigments were altered. SCHERTZ strongly advises using fresh leaves for all quantitative pigment extractions.

In the second experiment soy bean plants were grown thirty days (January 18-February 17, 1925) in Knop's solution supplied iron as ferrous sulphate at rates from 0.367 to 7.340 p.p.m. iron, as indicated in table I, and in the form of ferric citrate at rates from 0.228 to 4.560 p.p.m. as given in table II. Four cultures of four plants each were employed for each concentration of iron. The solutions were

renewed every five days. The total quantity of iron supplied in mg. per plant was 0.83 times the concentration in p.p.m. The initial hydrogen-ion concentration of the solutions varied from P_H 5.5 to 5.7.

The foliage of the plants in solutions 1 and 2 of both series became chlorotic. The black spot condition of the leaves was most pronounced in solutions 1, 2, and 3 of the ferrous sulphate series. Immediately upon determining the green weights of the plants, the pigments of 10 gm. samples of the fresh leaves were extracted with acetone.

TABLE I

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, FERROUS SULPHATE SERIES

	SOLUTION AND LEAF SAMPLE NUMBER				
	1	2	3	4	5
Concentration of iron, p.p.m....	0.000	0.367	1.835	3.670	7.340
Green weight in gm.....	7.705	10.423	13.158	13.651	14.925
Ether extract of chlorophyll and carotinoids.....	0.78	1.00	1.00	1.02	0.95
Chlorophyll (a and b)*.....	0.0045	0.0104	0.0134	0.0137	0.0142
Total carotinoids.....	0.55	1.00	0.64	1.00	0.74
Carotin.....	0.55	1.00	0.97	1.42	1.48
Xanthophyll.....	0.52	1.00	0.87	1.18	0.97

* Grams of purified chlorophyll (a and b) equivalent to the chlorophyll (a and b) from 10 gm. of fresh leaves.

The chloroplast pigments were transferred without loss from acetone to ether, and the relative values of the combined chlorophylls and carotinoids determined by comparison with those of sample 2 of each series. The chlorophyllin salts were secured and compared with an alcoholic solution of 0.257 gm. of purified chlorophyll (a and b) in 250 cc. The combined carotinoids, carotins, and xanthophylls were then compared relatively with those of sample 2. The values for these determinations are summarized in tables I and II.

Ferric citrate was again the most efficient source of iron, but in this experiment the order of efficiency was much higher than in the previous one. For solution 5 the iron of ferric citrate was 13.3 times as efficient as that of ferrous sulphate, for solution 4, 14.7, and for

solution 3, 12.2. The optimum concentration of iron as ferric citrate was 2.280 p.p.m., but with ferrous sulphate the highest concentration employed (7.340 p.p.m.) gave the most growth.

With both ferrous sulphate and ferric citrate an increase in the iron supply to the plants resulted in increases of the chlorophylls of a unit of fresh leaf material. An exact proportional relation did not exist between the iron supplied and the amount of chlorophyll found per unit of leaf material. A very good correlation appears to exist between the chlorophyll content per unit of fresh leaves and growth,

TABLE II

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, FERRIC CITRATE SERIES

	SOLUTION AND LEAF SAMPLE NUMBER				
	1	2	3	4	5
Concentration of iron, p.p.m. . .	0.000	0.228	1.110	2.280	4.560
Green weight in gm.	7.984	13.968	16.988	18.732	17.928
Ether extract of chlorophyll and carotinoids	0.38	1.00	1.26	1.53	1.60
Chlorophyll (a and b)*	0.0058	0.0087	0.0138	0.0160	0.0160
Total carotinoids	0.46	1.00	1.00	1.07	1.47
Carotin	0.22	1.00	1.11	1.21	1.53
Xanthophyll	0.34	1.00	0.96	1.21	1.49

* Grams of purified chlorophyll (a and b) equivalent to the chlorophyll (a and b) from 10 gm. of fresh leaves.

as can readily be seen from the curves of fig. 2. The values of the chlorophylls (a and b) in terms of purified chlorophyll (a and b) almost equal the growth of the plants when multiplied by 1000, except with the samples from plants grown without iron.

The values of the total chloroplast pigments in the ether extracts and the carotinoids present some relations that differ from the chlorophyll values. In the ferrous sulphate series the total chloroplast pigments from leaves of plants grown in solution 1 show a marked diminution, but those of solutions 2, 3, 4, and 5 give practically the same values. The leaves of the plants in solution 2 were light green, while those in solutions 3, 4, and 5 were normal green. Why this difference was not evident in the ether extracts cannot be explained. In the ferric citrate series the pigments in the ether ex-

tracts increased in their relative values from solution 1 to 5, which would appear logical judging from the values of the chlorophylls. The combined carotinoids of samples 2 and 4 of the ferrous sulphate series, and samples 3, 4, and 5 of the ferric citrate series exhibited a greenish cast which could not be removed by washing with distilled water, 1 per cent sodium carbonate solution, or by filtering through

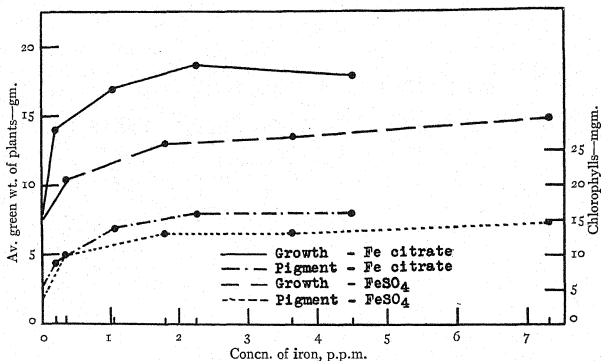


FIG. 2.—Green weights of soy bean plants per culture supplied four concentrations of iron as ferrous sulphate and ferric citrate, and amounts of chlorophyll (*a* and *b*) produced per 10 gm. fresh leaves; chlorophyll (*a* and *b*) expressed in terms of purified chlorophyll (*a* and *b*).

anhydrous sodium sulphate. This made it difficult to make accurate relative comparisons.⁴ The separated carotins and xanthophylls of both series exhibited increases with increased iron supply except in the case of sample 5, ferrous sulphate series, where a reduction was found.

Influence of potassium

Two experiments were performed on the influence of potassium on the growth, and on development of the chloroplast pigments of soy bean plants. In the first experiment the plants were grown

⁴ SCHERTZ suggested that the greenish cast of the carotinoid solutions might result from the incomplete saponification of the ether extracts.

thirty-six days (October 12–November 17, 1924) in Knop's solution lacking potassium and containing 28, 134, and 238 p.p.m. potassium. Solution 3 with a concentration of 134 p.p.m. potassium, the normal Knop's solution, was considered the control solution. Each solution was replicated with four cultures of four plants each. The solutions were changed every four days.

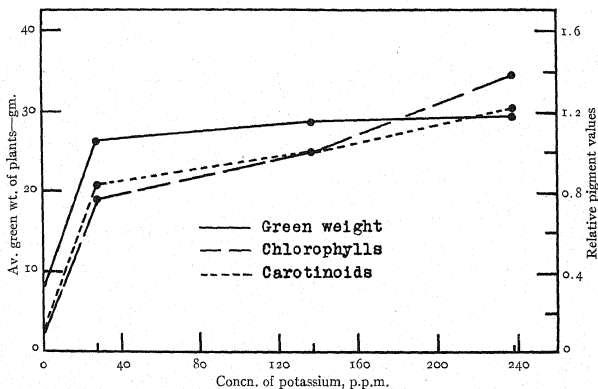


FIG. 3.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 2 gm. samples of dried leaves.

The growth of the plants in the solution lacking potassium was very much stunted, and the leaves developed large translucent areas on the blades and dark red areas along the stems. The plants in the solutions containing potassium made rapid growth. Only small increases in green weight were secured with additions of potassium above 28 p.p.m. (fig. 3). No differences in color of the leaves were observable between the plants in the low and high potassium-containing solutions.

The tops of the plants were air dried in the dark and the pigments extracted from 2 gm. samples of the leaves. In sample 1 some

difficulty was experienced in transferring all the green pigment to ether from the acetone extract. The relative values of the four chloroplast pigments, the chlorophylls, and the carotinoids in ether were determined by comparison with those of sample 3, which were given a value of 1.0. The chlorophyllins were also compared with a solution of 0.0025 gm. of purified chlorophyll (*a* and *b*) in

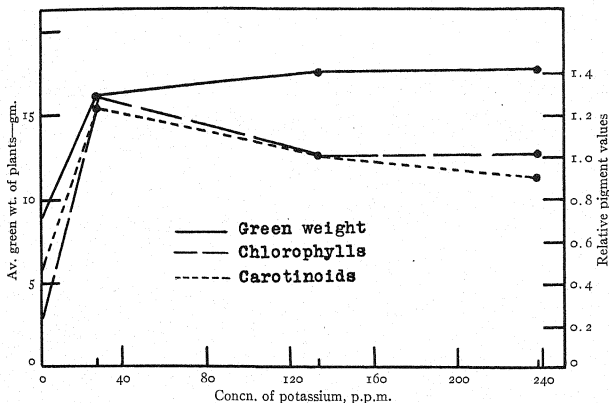


FIG. 4.—Green weights of soy bean plants per culture supplied three concentrations of potassium, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

100 cc. of alcohol. The results of these determinations are shown in fig. 3.

The chloroplast pigments of the leaves were greatly depressed when potassium was withheld from the plants, but the addition of 28 p.p.m. potassium to the nutrient solution resulted in a very large increase in these pigments. Rates of 134 and 238 p.p.m. potassium produced further increases in all the pigments except the xanthophyll of sample 4, which was lower than that of sample 3.

The extraction of the chloroplast pigments from the dried leaf samples in this case was apparently satisfactory. Observable loss of

the chloroplast pigments occurred only in sample 1. This was in distinct contrast to the results secured with the dried leaves of the series with iron. The difference can probably be accounted for by differences in the rapidity of drying of the two lots of leaves, although both were dried in the dark at room temperatures. Although no loss of pigment, with the exception noted, was observed in the wash water from the acetone extracts, the chlorophyll found in the dried leaves was much less than found later in fresh leaves. Expressing the chlorophylls in terms of purified chlorophyll (*a* and *b*), the maximum found in this series in 2 gm. of dried leaves was 5.3 mg., and in the second series with potassium in 10 gm. of fresh leaves the maximum was 18.0 mg. This would suggest a considerable loss of pigments during the drying process, assuming that the leaves contained approximately 80 per cent moisture.

The second experiment with potassium was a replicate of the one already described, and was performed to secure fresh leaves for the extraction of the pigments. The cultures contained four plants each. The solutions were changed every five days during a growing period of thirty days (January 14–February 13, 1925).

At the end of the experiment the plants in the solution lacking potassium were stunted, the leaves crinkled with large white areas in the blades, while some of the older leaves were turning yellow. Many of the stems exhibited dark red to brown areas. The new leaves of the plants in the solution containing 28 p.p.m. potassium were yellowish green and crinkled. The solutions with the highest concentrations of potassium, 134 and 238 p.p.m., produced plants that were normal in every respect.

The pigments of 10 gm. samples of the fresh leaves were extracted immediately upon harvesting the plants. The ether extracts containing the chlorophyll (*a* and *b*) and carotinoids, and the combined carotin and xanthophyll were compared relative to those of sample 3. The chlorophylls were compared with a solution of 0.0257 gm. purified chlorophyll in 250 cc. of alcohol. A summary of these determinations is given in table III.

The chloroplast pigments were markedly reduced when potassium was omitted from the nutrient solution, but showed a very great increase with a concentration of 28 p.p.m. of this element. The

values obtained for sample 2 appear to be too high when compared with those of samples 3 and 4, because at the time of harvesting the plants many of the leaves in solution 2 were slightly chlorotic, while those in solutions 3 and 4 were normally green. The series in which dried leaves were used for extraction also shows no such high chlorophyll content for the plants in solution 2. Duplicate samples of the fresh leaves in the second series gave similar high results for sample 2. The chlorophylls of sample 4 when compared with a solution of purified chlorophyll (*a* and *b*) were greater than those of sample 3, but relative comparisons of these pigments gave approximately the

TABLE III

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, SECOND POTASSIUM SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of K, p.p.m.	0.000	28	134	238
Green weight in gm.	8.995	16.178	17.722	17.756
Ether extract of chlorophyll and carot- tinoids.		1.20	1.00	0.95
Chlorophyll (<i>a</i> and <i>b</i>)	0.22	1.32	1.00	1.07
Chlorophyll (<i>a</i> and <i>b</i>)*	0.0030	0.0180	0.0136	0.0146
Total carotinoids	0.48	1.25	1.00	0.90

* Grams of purified chlorophyll (*a* and *b*) equivalent to the chlorophyll (*a* and *b*) from 10 gm. of fresh leaves.

same values. The carotinoids of sample 4 showed a slight decrease as compared with those of sample 3.

Influence of sulphur

Soy bean plants were grown in solutions lacking sulphur and containing 13, 26, and 46 p.p.m. of this element. Four plants were placed in each culture and the cultures replicated four times for each solution. The solutions were renewed every five days during a growing period of forty-three days (January 19–March 3, 1925).

A slight chlorosis of the new leaves of the plants in the solution containing the highest concentration of sulphur (46 p.p.m.) occurred in the fourth week, but by the end of the growing period these leaves were normal green. Some of the leaves were crinkled and developed

white to rust colored areas in the blades, together with a drying of the leaf margins. A similar crinkling of new leaves and discolored areas in the blades were also found in the plants deprived of sulphur. A swelling of lateral roots was noted in the plants lacking a supply of sulphur and those receiving the lowest concentration of this element.

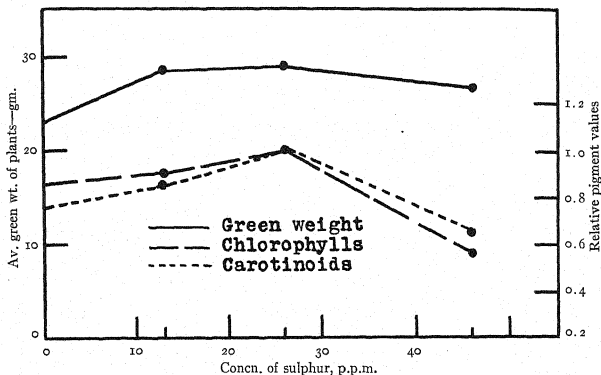


FIG. 5.—Green weights of soy bean plants per culture supplied three concentrations of sulphur, and relative values of chlorophyll (*a* and *b*) and carotinoids of 10 gm. samples of fresh leaves.

The growth of the plants was only slightly influenced by the concentration or amount of sulphur supplied in the nutrient solutions, as indicated by the green weight data of table IV. DUGGAR (5) states that sulphur is usually required in such limited quantity that the seeds may furnish all that is needed for normal growth of plants through a considerable period. HART and PETERSON'S (9) data on the sulphur content of a number of seeds show that soy bean seeds are high in this element. Soy beans contain 0.341 per cent sulphur, wheat 0.170, corn 0.164, and oats 0.189 per cent.

The chloroplast pigments of 10 gm. samples of fresh leaves were extracted and determined relatively. These data are given in table

IV. The maximum development of the chloroplast pigments per unit of fresh leaves was found in the plants grown in the regular Knop's solution (solution 3) with sulphur at a concentration of 26 p.p.m. A decrease or an increase in the sulphur supplied as compared with that of Knop's solution decreased the quantity of chloroplast pigments, the excess sulphur supply causing the greater depression.

TABLE IV

AVERAGE GREEN WEIGHTS OF SOY BEAN PLANTS PER CULTURE AND
RELATIVE VALUES OF CHLOROPLAST PIGMENTS OF 10 GM.
FRESH LEAVES, SULPHUR SERIES

	SOLUTION AND LEAF SAMPLE NUMBER			
	1	2	3	4
Concentration of S, p.p.m.....	0.000	13	26	46
Green weight in gm.....	23.340	28.517	28.959	26.949
Ether extract of chlorophyll and carot- inoids.....	0.71	0.83	1.00	0.58
Chlorophyll (a and b).....	0.86	0.90	1.00	0.57
Chlorophyll (a and b)*.....	0.0092	0.0094	0.0107	0.0058
Total carotinoids.....	0.76	0.86	1.00	0.65
Carotin.....	1.03	0.81	1.00	0.46
Xanthophyll.....	0.98	0.80	1.00	0.46

* Grams of purified chlorophyll (a and b) equivalent to the chlorophyll (a and b) from 10 gm. of fresh leaves.

Discussion

Of the three mineral elements studied, the lack of iron was found to limit the growth of soy bean plants most, followed by potassium. Sulphur had a relatively slight influence on the growth of these plants. The source of the iron presented some interesting features in connection with growth. Ferric citrate in the first experiment proved to be about four times as efficient a source of iron as ferrous sulphate, and in the second experiment about thirteen times as efficient. The difference in the value of this factor for the two experiments is probably due to differences in environment, and to the fact that one series was grown for five weeks and the other for four weeks. The relative constancy of this factor of efficiency in each of these experiments indicates a constant difference in the availability of iron in the citrate and sulphate. The cause of the difference in efficiency of the iron in these two salts may be due to a lower solubility of the

ferrous sulphate, the ferric or ferrous state of the iron or the anion with which the iron is combined. Of these possible causes, that of solubility appears to be the most probable. TOTTINGHAM and RANKIN (23) found that the solubility of iron as ferric citrate was about four times that of iron as ferrous sulphate in the Livingston-Tottingham solution R_8C_1 at a P_H of 6.0. A similar constant relation can also be found in the data of JONES and SHIVE (10) with ferric phosphate and ferrous sulphate in the growth of wheat. Ferrous sulphate was approximately 1.6 times more efficient than the less soluble ferric phosphate.

The optimum concentrations of ferric citrate were 2.280 and 3.648 p.p.m. iron, but the highest concentrations of ferrous sulphate employed (7.340 and 8.822 p.p.m. iron) produced no decrease in growth, so the optimum concentration was not attained. MARSH and SHIVE (15) obtained the highest yield of soy bean plants with ferrous sulphate at the rate of 19.4 mg. iron per liter for three plants, or 6.4 mg. per plant over a growing period of five weeks when the iron supply was adjusted at frequent intervals. In the present experiments a supply of 13.233 and 6.116 mg. iron as ferrous sulphate per plant, over growing periods of five and four weeks respectively, gave very satisfactory growth.

The value of the determination of the chloroplast pigments depends upon the reliability of the methods used for such determinations. The operation is tedious and complicated, and there are numerous opportunities for error. It was found that the pigments of fresh leaves could be extracted much more easily and completely than those from dried ones. The acetone extracts from dried leaves often contained water-soluble green pigment which could not be transferred to ether. Determinations of the separated chlorophyllins from such extracts gave values that were obviously too low, which indicated a decomposition of the pigments during drying. Relative comparisons of 80 per cent acetone extracts of the total pigment content of leaf samples were not satisfactory, because of the presence of water soluble pigments. A comparison of the total chloroplast pigments by transferring from acetone to ether and washing out all water soluble material has the double disadvantage of permitting relative determinations only and of including both yellow and green

pigments. The comparison of the chlorophylls with known solutions of chlorophyll (*a* and *b*) gave the most satisfactory results, because the colors were comparable, and because, while not expressing exactly the quantity of chlorophyll present, it did allow a comparison of the amount of these pigments among different experiments. The carotinoids combined and separated were particularly difficult to determine accurately. A greenish tint impossible to remove in some of the carotinoid extracts made relative comparisons difficult. The cause of this variation in color could not be determined. The addition of concentrated sulphuric acid to a carotinoid extract in petroleum ether produces a clear green color, the original yellow color returning upon dilution with water. It is possible that a partial change of the carotinoids to this greenish form may occur during some stage in the manipulation. SCHERTZ is of the opinion that the greenish tint is the result of incomplete saponification of chlorophyll (*a* and *b*) altered probably by drying. Nevertheless, certain facts of interest appear evident from the determinations of the chloroplast pigments. The omission of iron or potassium from the solutions reduced the amount of the chloroplast pigments decidedly more than the omission of sulphur. This, however, does not mean that if sulphur were seriously deficient the pigment production would not be affected. The relatively high sulphur content of soy bean seeds probably supplies the seedlings with enough for a considerable period. The general tendency was for the pigments to increase with increasing supplies of iron and sulphur, but with the highest concentration of sulphur (46 p.p.m.) a very decided decrease in pigment content resulted. Judging from the results secured from the fresh leaves, the higher concentrations of potassium also caused a decrease in the amount of the chloroplast pigments.

One rather unexpected feature of the analyses is the fact that the carotinoids and chlorophylls vary for the most part together. A reduction in the chlorophylls is associated with a similar reduction in the carotinoids. This suggests that the formative processes involved in the production of these two types of pigments are influenced similarly by the essential elements included in this study. It also suggests that the yellowish colors frequently observable in chlorotic leaves are not due to carotin or xanthophyll but to xan-

thones, flavones, or related yellowish pigments. There is no evidence from the data to indicate that the formation of the chlorophylls is more dependent upon iron, potassium, or sulphur than the formation of the carotinoids. On the contrary, the determinations emphasize the close relationship which appears to exist between these two groups of chemically different pigments.

Summary

1. Soy bean plants were grown in nutrient solutions in which the concentrations of the iron, potassium, and sulphur contents were varied.

2. Ferric citrate was found to be four to thirteen times more efficient an iron source than ferrous sulphate. This difference in efficiency was constant in a given experiment.

3. Small differences in the iron content of the nutrient solutions produced considerable differences in the growth of the plants.

4. Iron deficiency caused small black spots to develop in the chlorotic new leaves.

5. Lack of potassium stunted growth markedly, but concentrations of 28-238 p.p.m. of this element produced about the same amount of growth.

6. The sulphur content of the nutrient solution influenced growth only slightly.

7. Acetone extracts of the total pigments of leaves are not safe criteria for judging the relative chloroplast pigment development.

8. Ether extracts of the chloroplast pigments freed from water soluble pigments are more satisfactory for judging the relative chloroplast pigment content than acetone extracts.

9. The separated carotinoid pigments may show greenish tinges which interfere with their quantitative estimation.

10. Estimation colorimetrically of chlorophyll (*a* and *b*) against a standard of purified chlorophyll (*a* and *b*) in alcohol was found to be a very satisfactory method.

11. The lack of iron or potassium in the nutrient solution resulted in a more marked depression of the chloroplast pigments than a lack of sulphur.

12. In general, the chloroplast pigments increased with the in-

creasing concentrations of iron and sulphur. The highest concentration of sulphur used (46 p.p.m.) caused a marked decrease in the pigment content of the leaves.

13. Concentrations of potassium above 28 p.p.m. reduced the chloroplast pigments when the extractions were made from fresh leaves.

14. In all the experiments the chlorophylls (*a* and *b*) and carotinoids were influenced by the composition of the nutrient solution to about the same extent.

15. An exact proportional relation could not be established between the concentration or amount of any of the elements studied and the amount of pigment formed.

16. A correlation between the green weight of the plants and the chlorophyll (*a* and *b*) existed in one experiment with iron, but did not apply in the other experiments.

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TRANSLOCATION OF FATS AS SUCH IN GERMINATING FATTY SEEDS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 351

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Introduction

Among the problems that challenge our interest in connection with the translocation of foods, the one which has proved most baffling to the investigator is that of explaining the forces responsible for the movement of solutes from cell to cell through long distances in the plant. No generally satisfactory explanation has yet been proposed, although several have been suggested. This is true of the soluble compounds, but it would be much more difficult to explain the movement from cell to cell of insoluble bodies, granules, or droplets, of which the fats are an example. Indeed the propulsion of fat droplets through the plant, if this occurs, is a striking question in plant dynamics.

The statement that fats can move as such from cell to cell is frequently found in the literature, and usually specifies that they probably move in the form of "fine emulsions." A canvass of a limited circle of plant physiologists revealed the fact that there existed great uncertainty on this point, some thinking the fats moved as fats in the emulsified state, others supposing that they moved as fatty acids and glycerol, and still others believing there was not enough evidence on the subject to justify a statement. It appears that no actual work on this problem has been reported in the last thirty-five years. The older work is cited in support of fat movement in such standard texts as those of PFEFFER, JOST, and BENECKE-JOST, and those on plant chemistry by CZAPEK and by HAAS and HILL.

SACHS (4) in 1859 observed the presence of considerable quantities of fat in the hypocotyls of germinating fatty seeds, and was led to the conclusion that it had moved out from the seed in that form. His *Physiology of plants* of 1889 contains the following statement of

his position: "It thus appears that the fats can pass the closed tissue-cells as such; though of course the greater part of them is transformed to starch and sugar for transport and use." SCHMIDT (5) cites PETER's work as supporting SACHS' claim by quantitative determination of the fat in the hypocotyl. PETER, too, thought the fats moved as fats. SCHMIDT, working in PFEFFER's laboratory in 1890, arrived at the same conclusion from similar observations.

SCHMIDT, however, went much further in his extensive experiments. He planned elaborate tests to see whether the living cell could take up oils from the outside. First he tried the fungi, and, although he was apparently successful, he did not consider the results conclusive, or applicable to the higher plants. Next the mosses were used, but here it was found necessary to dry the moss leaves for eight days at 28° C., and then use a partial vacuum in order to get the oils to penetrate the walls. This was not analogous to the situation obtaining in the tissues in which he thought the oils moved, and he did not consider it as evidence. Finally, SCHMIDT tried the injection of oils into etiolated starved pea seedlings, thinking that in this case the intake of oil by the cells would be evidence of the possibility of fat movement from cell to cell in the normal plant. His method consisted in growing pea seedlings in the dark until the food was exhausted from the cotyledons (parts of which were cut away to hasten starvation). A longitudinal incision was then made just above the ground, and a strip of filter paper, saturated with the oil to be used, was inserted in the incision. If colored oils were used the rise and descent of the oil through the intercellular spaces could be observed. By a very difficult technique and by using longitudinal sections, the presence of oil drops within the cells could be determined with a moderate degree of certainty. His results may be stated briefly as follows: Using almond oil and oleic acid, he found that the more acid there was mixed with the oil, the more rapid the rise. With pure fatty acid the cell was penetrated and droplets were found within the cytoplasm after some hours' standing. SCHMIDT reports that the neutral oil did not enter the cell unless there was at least 10 per cent free acid present. This led him to propose an explanation which involved the formation of soaplike compounds with the free acid and the emulsification of the neutral fat by these soapy

substances. He thought it passed through the wall as a "fine emulsion," and assumed from this that the fats could pass from cell to cell.

SCHMIDT also studied germinating fatty seeds. His most important evidence for fat movement from these studies is the fact that he found no sugar or starch in the endosperms during germination. He assumed from this that the fats do move as fat from the cells of the endosperm to the cotyledons.

This is the case for fat movement, so far as could be ascertained from the literature. No evidence was reported against it. The essential points of SCHMIDT's work have been repeated, and then further experimentation was undertaken with germinating fatty seeds, in the hope of finding a satisfactory basis for the solution of the problem. The results of both phases of the work are reported here.

Investigation

The first point to be investigated was the reported intake of fats by the cells of etiolated pea seedlings. Seedlings were grown until they were in a starved condition, incised longitudinally, and a strip of filter paper saturated with oil colored with Sudan III was then inserted. The oils used were linseed, linseed fatty acids, olive oil, oleic acid, and castor oil, giving a wide range of viscosity, as may be seen in table I, taken from LEWKOWITSCH (2).

TABLE I
VISCOSITY OF CERTAIN OILS AT 15° C. (SCHÜBLER)

Oil	Viscosity
Castor oil	203.3
Olive oil	21.6
Almond oil	16.6
Linseed oil	9.7
Distilled water	1.0

Almond oil was not used, but its place in the viscosity range may be seen in the table. Following SCHMIDT's procedure, the seedlings were tested with the various oils, and, using the dissolved stain as an indicator, the rate of rise of each in the stem was observed. Table II gives the results.

The movement of the oils was through the intercellular spaces, and was confined mainly to the outer regions of the cortex. In some cases the oil would get into the tracheae and seemed to be drawn up by the pull of transpiration. Such cases were disregarded.

TABLE II
RISE OF OILS IN PEA STEM

OIL	RISE IN CM. PER HOUR
Castor oil.....	0.05
Olive oil.....	0.50
Linseed oil.....	2.00
Oleic acid.....	0.75
Linseed fatty acids.....	1.00

It will be noticed that, contrary to SCHMIDT's findings, the fatty acid in the pure state did not rise as rapidly as did the neutral fats of these fatty acids. Also, the more viscous of the neutral fats did not rise as rapidly as did the less viscous. The fact that the free fatty acids did not rise as rapidly as the neutral fats might perhaps be due to their chemical properties in part, and to a different force of adhesion resulting from the chemical difference; but the reason for this difference in behavior is not clear. It may be mentioned here that these fatty acids, when applied to leaves of geranium, tomato, begonia, etc., kill the tissue with which they come in contact within twenty-four hours, while the neutral oils do not. Small amounts of neutral oil applied to one side of the leaf did not injure it, although large amounts naturally clogged all the openings of the leaf and injury resulted.

Most important, of course, was the intake of the oils by the cells. The fatty acids entered the cytoplasm from the intercellular spaces and appeared in the cytoplasm in the form of small droplets, which could be distinguished from those just outside of the cell by SCHMIDT's method of plasmolyzing the cell or by finding the oil in cells which happen to have rotating protoplasm. So far as intake of free fatty acids was concerned, therefore, SCHMIDT was evidently correct, but it was found that linseed oil with a free acid content of less than 2 per cent penetrated the cell as well as the free acid. It was evident, therefore, that these results again failed to check with those of SCHMIDT, who claimed that neutral oil did not penetrate

the cell. It seemed likely from this that the penetration was a physical matter, depending in part at least upon viscosity of the oil, and not a chemical process as he supposed.

The penetration of the cell wall by the oils being established, the next point to be investigated in the case for fat movement which SCHMIDT had made, was the soundness of the assumption that their passage from the intercellular spaces into the cell justified the conclusion that the fats could pass in a similar way from one cell to another. This point was tested on two bases: (1) Since the fat in the plant cells during translocation is in small droplets (emulsified), while the fats taken from the outside by the cells of the pea seedling are pure and homogeneous, the cases could not be considered at all comparable. Intake must be demonstrated from the emulsion state, with the continuous phase a water solution. (2) Since the seedlings used in SCHMIDT's experiments were starved and etiolated, an abnormal condition, it should be ascertained whether any alteration in the condition of the walls bordering on the intercellular spaces might have been brought about, such as degree of saturation, that would be different from the conditions existing in the germinating fatty seed.

In order to test the first point in question, emulsions of linseed fatty acids were prepared with water, the fatty acids forming the dispersed phase. After three days' standing in this emulsion, the pea seedlings showed no intake whatever of the fat by the cells. Seedlings were incised in the usual manner, and the emulsion brought into contact by means of a jacket slipped over the pea stem. No rise of fat in the intercellular spaces or intake of fat by the cells was detected. It appeared, then, that when the plant conditions were approximated in this matter, the fats failed to penetrate to the interior of the living cell.

On the second point a suggestion was given by the results on the first, namely, that the presence of water might interfere with the intake of fat from the intercellular spaces; that is, that the penetration of the cell walls by the oils was due to a water deficit. It is well known that oil will not pass through cellulose membranes that are saturated with water, but will penetrate as the water dries out. SCHMIDT went into this phase very thoroughly, testing also some

plant membranes, with the same general results. A very definite suggestion that this might be the explanation of the intake of the oil lay in the fact that the cells which took up the fat were located in the periphery of the stem. The epidermal and subepidermal cells showed the most penetration. The intercellular spaces are large, and they connect with the outside through the stomata. This connection is easily demonstrated by placing a drop of colored oil on the pea stem and noting that it rises up through the internal spaces after half an hour. Consequently, we should expect that this etiolated, uncutinized, delicate seedling would lose water readily, especially from the cells nearest the epidermis. In fact, when the stored foods have been exhausted and the plants are exposed to a dry air in the laboratory, the epidermis of the stems soon wrinkles with water loss. Probably the starved condition interferes with its normal water holding powers.

With this situation in mind, the following test was made to discover whether water deficit played any part in the results obtained by SCHMIDT. Etiolated pea seedlings were placed under a bell jar and the soil was soaked with water; the peas and the air were soon approximately saturated. Another group of seedlings, in dry soil, was placed under the current from an electric fan. Then the seedlings were incised and the oiled strips of filter paper inserted. After two and four hours the amount of rise shown by the oil was noted in each case, and the cells were examined for penetration. In the seedlings saturated with water, the rise of the oils in most cases was zero, and of course then there was no penetration. In the few cases where some rise occurred, there was no detectable intake of oil by the cells. The increased drying effects of the air current increased the rate of ascent of the oil, however, and facilitated its penetration. Seedlings that were cut off, and, after injecting with oil, laid out to dry, showed a relatively rapid movement of the oil, while cut-off seedlings that were kept in water with a large test-tube inverted over the top permitted little or no rise. In this manner it was experimentally demonstrated that the intake of oil by the living plant cell, obtained by SCHMIDT, was due to an unnatural drying out of the cell walls, a condition not obtained in the tissues of the germinating fatty seed through which the fats have been supposed to pass as fats, if

indeed it obtains anywhere in the normal plant. The main point upon which the case for fat movement in the plant has rested, therefore, appears to have been reached through an abnormal condition of water deficit and through the use of oil in the pure instead of the emulsified state. Neither of these conditions exists in the plant in those tissues through which the oil has been thought to move.

Another point on which SCHMIDT had in a measure based his conclusion that the fats do move in the germinating fatty seed, was the fact that he found no sugar or starch in the endosperms of some of the fatty seeds during germination. When this point was investigated for the purpose of checking his results, it was found that there are large quantities of non-reducing sugar in the endosperm of germinating castor bean, as well as in hemp. A reducing sugar was found in flax endosperm during the germination phase. Others were not tested, but it was evident that SCHMIDT's report was in error, possibly because he may not have tested for non-reducing sugars.

Finally, there remained the oldest and most frequently cited evidence for fat movement in plants, the fact that in germinating fatty seeds oil is found in the hypocotyl, and, as SCHMIDT demonstrated, this oil in the new tissue is similar in composition to that of the storage region. The fact that oil was stored in the seed, and later, during germination, appeared to be present in the new organs, was taken as evidence that it moved from the point of storage to the point of utilization in the fatty state. This is not a logical conclusion. No one believes that starch grains move from cell to cell as starch, and yet a perfect case for starch grain movement could be based on this principle. Various starchy seedlings show starch in the hypocotyls and other new tissue, and there are even cases, such as the storing of foods in ripening seeds, which furnish the appearance of a gradient of starch flowing toward the center of storage. Examination of any of the fatty seeds available showed considerable fat in the hypocotyls during germination, but this could not be taken as evidence of movement as fat. Similarity in the composition of the fat in the seed and the hypocotyl again has its parallel in the similarity in the composition of the starch in the seed and in the new organs; yet we do not conclude from such comparisons that starch moves as starch. The plant has its "patterns," and the like protoplasm of the different regions merely follows these.

SCHMIDT's theory of "soaplike linkages" operating in the intake of fats by the cell should be mentioned, in view of the fact that it is believed by some today that fatty acids may be transported as soaps. For this to be possible the tissue must be alkaline, in order that the soap may not be decomposed. Seedlings of a number of the fatty seeds available were tested microchemically with the LaMotte series of indicators, and in all cases all the new tissues were found to be acid. The highest P_H found was 6.8, and in most of the tissues it was below 6.2. These determinations are accurate to within 0.2 in the P_H reading, which is sufficient for this problem. Furthermore, it seems to be the only method available by which local H^+ ion concentration may be determined in the tissues. Table III gives the ranges found in the hypocotyls of the seedlings tested. The root tips, both primary and secondary, were nearest the neutral point but were not above 6.6.

TABLE III
RANGE OF H^+ CONCENTRATION IN HYPOCOTYLS

SEED	RANGE OF P_H
Peanut (fatty).....	5.4-5.8
Sunflower (fatty).....	5.2-5.6
Castor bean (fatty).....	5.2-5.6
Hemp (fatty).....	6.2-6.6
Pea (starchy).....	5.6-5.8

The phloem of these tissues was examined with special care, in view of the fact that it has been said to be alkaline (4), but it was no exception to the general rule of acidity in these seedlings. Under these conditions, of course, it would be difficult to conceive of the existence of, and still more the movement of, soaps in the plant.

This concludes the report of the results obtained by attempting to repeat work previously done on the subject of fat movement. When the past evidence in favor of the transport of fats as such is repeated and reinterpreted, it is found that there is not even a legitimate suggestion that such movement occurs in the plant. In fact, there is no acceptable evidence as yet reported on either side of the question. For this reason further experimentation was carried out on the problem, with a view to finding some substantial and definite evidence leading to a solution. The results given here are not as extensive as might be desired, nor is the attack on the problem

as varied as might be, were the problem more easily approached. All evidence obtained, however, whether reported or not, pointed in the same direction, and only that is reported which is thought to be incontestable in its application.

The first attack on the question was made from the standpoint of the respiratory quotient, the object being to determine the type of food used by means of the type of respiration of the new tissue. It is well known that when sugars are being respired, a definitely higher CO_2/O_2 quotient is obtained than when fats are being respired. Theoretically the quotient ought to be about 1.0 for the carbohydrates in normal conditions, and about 0.67 for fats, but under the rapid growing conditions that prevail in germination the quotient is lower than normal, probably due to the formation of organic acids. If any considerable portion of the fat of the seed were to be moving into the hypocotyl during germination, then the tissues of the hypocotyl would be digesting and respiring fat, in part at least, and the respiratory quotient would accordingly be lowered. Whereas, if the hypocotyl received only sugars, that is, if the fat of the seed moved into the hypocotyl as sugar, we should expect to find a respiratory quotient of the same order as in the hypocotyls of starchy seeds. Even though the sugar was only the transit form, and was converted back to fat immediately upon reaching a certain point in the new tissue, the quotient would be a carbohydrate quotient, since the temporary storage of the sugar in the form of fat would be cancelled out or balanced off by the corresponding transformation of this temporary fat back to sugar for utilization. In short, if the carbon stored as fat in the seed should move as sugar into the hypocotyl, it does not matter what changes back and forth it may go through before utilization, so far as the effect on the respiratory quotient is concerned; a quotient should be given of the same order as that of the starch-storing seed.

The method and apparatus used in determining the respiratory quotient of this material are modifications of those used by RHINE (3). The bottoms of three wide bottomed pint fruit jars were covered with wet cotton, and fitted with rubber stoppers, in which were set a mercury manometer and an outlet tube covered with heavy rubber tubing and closed with a screw clamp. The first jar was left with

only the wet cotton, as a check on temperature and barometric changes. The second was fitted with a small flat vat holding 10 ml. of 20 per cent NaOH, and around this vat the seedling material was laid. In the third was placed a like amount of plant material. In the second and third, the seedlings were covered with moist cotton, and all the jars were effectively sealed by a heavy application of sealing wax, completely covering the stopper and the connections with the tubing. The chambers were then placed in a water bath and allowed to come to the temperature of the bath before closing the outlet tubes. A water bath is not necessary with this method, however; it is merely convenient.

In some cases the hypocotyls were cut off, and in others the attempt was made to inclose the storage region or seed proper in a thick coating of beeswax or paraffin, and thus smother its respiration so that it would not affect the air of the chamber. Other substances were used also, but in general the results were best when the hypocotyls were simply cut off, paired carefully, and one lot of 50 or 100 put in each of nos. 2 and 3. When the seeds were coated there was danger of the coating being imperfect, and allowing the respiration of the storage region to affect the reading; but it must be remembered that any such error would throw the readings in the direction that would have opposed rather than favored the conclusion drawn from them. The results of these determinations are stated as respiratory quotients, and, since jars of equal volume were used, could be computed from the manometers. The data are given in table IV.

The very considerable variation in the results is due to variety in the tissues used; it is not possible to get exactly similar conditions in the samples used in different tests, and the value of the data will necessarily depend upon the number of determinations made. The average is the significant figure; the range of experimental error lies within $\pm .025$, although the variation of the tissue is greater than this figure. There are two sets of readings from fatty hypocotyls giving a quotient of 1.0 which are too high to be typical. While they would be favorable to the conclusion that is drawn from these data, they should not be included in the averaging of the results. Also, quotients as low as 0.66 have been obtained on pea (starchy) hypo-

cotyls, but these are unusual, and, though they would favor the conclusion based on the general data, have been eliminated. Other-

TABLE IV
RESPIRATORY QUOTIENTS ON HYPOCOTYLS OF FATTY AND STARCHY SEEDS

NAME	AVERAGE LENGTH (CM.)	TREATMENT	NUMBER OF READINGS	RESPIRATORY QUOTIENT
Fatty seeds				
Cotton 1.....	4	Cut off	2	0.80
2.....	4	Coated beeswax	4	0.73
3.....	3	Coated paraffin	2	1.00 (disregarded)
4.....	3	Coated paraffin	1	1.00 (disregarded)
Sunflower 5.....	3	Coated beeswax	3	0.90
6.....	3	Coated beeswax	3	0.75
7.....	4	Coated beeswax	2	0.74
8.....	4	Cut off	2	0.75
9.....	4	Cut off	2	0.70
10.....	5	Cut off	1	0.77
11.....	4	Cut off	1	0.82
12.....	5	Cut off	3	0.75
Average of total readings (except 3 and 4)				0.770
Starchy seeds				
Pea 1.....	4	Cut off	2	0.70
2.....	3	Cut off	2	0.66 (disregarded)
3.....	6	Cut off	2	0.85
Wheat embryos 4....	1.5	Cut off	2	0.77
5.....	2	Cut off	2	0.78
6.....	2	Cut off	2	0.75
Barley embryos 7....	4	Cut off	3	0.74
Buckwheat 8.....	3	Cut off	4	0.80
Average of total readings (except 2).....				0.775
Respiratory quotients on cotyledons alone				
Sunflower 1.....	3 days old	Cut off	1	0.33
2.....	4 days old	Cut off	1	0.58

wise all the results that are free from errors in manipulation have been averaged.

It is apparent from the evidence of respiration that the food supplied to the hypocotyls by the storage region of fatty seeds reaches them in a form yielding the same order of respiratory quotient as

does the food supplied by the typically carbohydrate storage seeds. This is commonly understood to be sugar, and the great amount of sugar present in seedlings of the starchy type is considered as indication that this is the transportation form. It would seem, therefore, that fat could not be moving into the hypocotyl as fat, at least in such quantity as to affect the respiratory quotient.

The problem was then attacked from another angle, that of gradients. If it were possible for fats to move in the insoluble state (as droplets, fine emulsions) from the storage to the growing point of the hypocotyl or root tip, we might expect to find them in a "diffusion" gradient of concentration from the source out to the point of utilization. That is, the movement of these droplets could hardly be rapid in any case, and in view of the rapid rate of growth it seems reasonable that the movement of fats as such would provide a gradient of decreasing concentration going toward the region of active growth. To secure evidence on this point, both microchemical observations and quantitative determinations were made on hypocotyls of fatty seedlings. Using the microscopic methods with Sudan III, the hypocotyls of cotton, sunflower, squash, cocklebur, castor bean, peanut, and flax, taken when 1-6 cm. in length, were studied. In no case was there a gradient from the storage region to the growing tip. Strikingly to the contrary was the fact that in every seedling examined there was a distinct gradient in the other direction; that is, the amount of fat increased with approach toward the tip of the hypocotyl. In fact, the tips were heavily laden with fat, although they were undergoing rapid elongation.

The results of the quantitative determination of the fats are given in table V. They represent the ether extract, and are stated as percentage of wet weight, since this is the most accurate basis for such a comparison, approaching most closely to a volume basis. The hypocotyls were severed from the seed just outside the seed coat, and were divided into three parts, tips, middles, and bases. Care was taken to get rid of all adhering moisture without losing water by evaporation. The cut parts were kept in closed weighing bottles in order to prevent loss of water.

It might be thought that there could be a local channel through the hypocotyl in which the fats might move, and yet not be present

in such quantity as to register a gradient. The solution of this question lay in the microscopical technique. No such channel was detectable, although fine droplets of fat were clearly evident in the cortical parenchyma. These were fewer in the middles and bases than in the tips, and as the quantitative figures on squash show, there can be as much as twelve times as much fat in the tip as in the base, although the base is close to the fat storage center.

A third approach to a solution of the question of fat movement was made through a thorough study of the new tissues in which oil droplets were found, using the common fat stains, Sudan III, Scarlet Red, etc., with a view to seeing whether any test for oil could be obtained in the walls of the cells through which the droplets would have to pass if such movement occurred. It was thought prob-

TABLE V
PERCENTAGE OF ETHER EXTRACT IN PARTS OF FATTY HYPOCOTYLS

SEEDLING	PERCENTAGE ETHER EXTRACT ON WET WEIGHT BASIS		
	Tips	Middles	Bases
Cotton.....	5.75	2.24	1.33
Sunflower.....	5.31	1.34	0.66
Squash.....	2.59	0.60	0.22

able that if fat were found in quantity in two cells that are adjacent, and if this fat were passing from one cell to another, there would be some fat necessarily in the walls "en route," in some instances at least. The tissues through which the carbon stored as fat in the seed have to pass in the seedling were carefully examined, both in cross and in longitudinal section, but no evidence of fat passing through the walls was seen, and no staining of the wall with fat stains was obtained. This method of attack cannot be stated very effectively, and the results cannot be put very convincingly, but it should be considered as more evidential than any point yet made, following in importance only the statement of results under the next method of approach. It should be mentioned here that the fatty compound obtained from cell walls by HANSTEEN-CRANNER (1) was obtained only after considerable hydrolysis, and its fatty character could hardly have been present before hydrolysis; in fact, the walls did not stain with fat stains.

Finally, the question was considered from a physical standpoint, the mechanics involved in fat movement. Since the hypocotyls grow downward the fat would have to move downward to reach the tip, where most of it is found. In view of the relatively high viscosity of the cytoplasm, especially in the first centimeter of the tips, this becomes a questionable proceeding, no matter how small the droplets, so long as they are not in true solution. The most important point in this connection, however, should be the relative densities of the fat and the cytoplasm down through which it would have to pass. The cytoplasm, being a hydrosol, can safely be assumed to have a specific gravity greater than 1.00; probably it is much greater, as solutions go. Extracts of the seedlings were made from different parts of the hypocotyl, and while there was not enough obtained to determine accurately the specific gravities of the various extracts, in every case they were lighter than water. They were less dense than egg white, and various concentrations of gelatin. There can be no doubt that they were lighter than the cytoplasm. It is true, as has been found in the course of some unpublished work on the oxidation products of fatty acids, that the highly unsaturated fatty acids readily become heavier than water through oxidation. The extract from the tips seemed most to resemble the oxidized oil, but even this did not become as heavy as water. Fat movement, then, would have to take place against the pressure of a denser medium, if it occurs. It will be remembered, of course, that SCHMIDT's movement of oils up and down the etiolated pea seedling was a movement of homogeneous oil, not an emulsion of oil in water, and that the movement was in the intercellular spaces, not through the living cells in any case.

Summary

1. It was found, as SCHMIDT had reported, that liquid fats would rise in the intercellular spaces of etiolated pea seedlings and would enter the cell. Contrary to SCHMIDT, however, it was found that neutral fats rose more rapidly than free acids in certain oils, and furthermore that neutral fats entered the cells from the intercellular spaces. SCHMIDT's theory of fat intake was thus shown to be incorrect.

2. It was shown that while the liquid fats pass into the cell from the intercellular spaces of the pea, this cannot be taken as analogous

to the situation existing in plants in which fats have been thought to move from cell to cell. It was shown that the intake of the fat was due to a water deficit in the walls of the starved etiolated pea seedlings, when they were exposed to unsaturated air, and that when well supplied with moisture in air and soil they would not allow either the rise or intake of oils.

3. SCHMIDT's report that the endosperms of fatty seeds did not have sugar during germination was found to be incorrect, and, therefore, this evidence is not admissible in support of fat movement in plants.

4. The presence of oils of similar composition in the new tissue and the old was shown to be of no significance in this connection, since the same condition prevails with respect to starch in starchy seedlings.

5. SCHMIDT's theory of "soaplike" linkages forming and aiding in fat movement is shown to be untenable, in view of the P_n values found in the seedlings investigated. This point also covers the question of movement of fatty acids as soaps, making the existence of soaps in the cell seem impossible.

6. By determining the respiratory quotients on hypocotyls of fatty and of starchy seeds during germination, evidence was obtained that the fatty seed hypocotyls were being furnished carbon in the same state of reduction as were those of the starchy seeds. The logical inference is that that form could not be fat.

7. If fats were to move as such a gradient of decreasing concentration might be found in the region of most active growth. The contrary is the case, both microscopical and quantitative data showing that a steep gradient exists in the opposite direction, increasing with approach toward the tip of the hypocotyl.

8. Cell walls in tissues through which the fats have been thought by some to pass, and in which fatty droplets may be found, have been examined for fats "en route." No evidence of such movement was found.

9. Since the oils would have to pass downward through several centimeters of denser viscous medium, to say nothing of the admitted impenetrability to oil of the water saturated cellulose membrane, the fat movement theory, from the standpoint of the physics in-

volved, would have to be given up, at least until some good positive evidence that such movement can and does occur is presented.

10. All evidence in favor of fat movement in plants that has been considered and reinvestigated has been found incorrect in fact or interpretation. The further evidence bearing on the problem, presented in this paper, favors the view that all the fat stored in the fatty seed is, as we have known most of it to be, first converted to sugars before being transported.

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PLANT SUCCESSIONS ABOUT DOUGLAS LAKE, CHEBOYGAN COUNTY, MICHIGAN

FRANK C. GATES¹

(WITH THREE FIGURES)

Introduction

During the period between 1911 and 1925, the writer has been making a detailed study of the region about Douglas Lake, the location of the Biological Station of the University of Michigan. During this study special attention has been paid to the successions between the various plant associations. The present paper is the result of that study.

Douglas Lake is located in the west-central part of Cheboygan County, at the northern end of the lower peninsula of Michigan, midway between Lakes Huron and Michigan, at an elevation of about 712 feet above sea level. The region which has been more thoroughly studied includes the area from Brutus east to Topinabee, north around Mud Lake and west to Levering, then south, with an extension west of Pellston, to Brutus (fig. 1). In addition, the region along Lake Michigan from Cecil west to Temperance Point has received special study. The surface soil is entirely of glacial origin.

According to the scheme of life zones drawn up by MERRIAM,² the Douglas Lake region would be considered as belonging to the Canadian part of the boreal zone, although it actually partakes of transition zone characteristics as well. According to the moisture-temperature index of LIVINGSTON,³ the 3300 calculated for Alpena, a short distance south, indicates an area of relatively low plant development. Agriculturally, therefore, the region would be considered

¹ Paper no. 205 from the Botanical Department of Kansas State Agricultural College. For the higher plants of the region see GATES, F. C., and EHLERS, J. H., An annotated list of the higher plants of the region of Douglas Lake, Michigan. *Michigan Acad. Sci., Arts and Letters* 4:83-284. 1924.

² MERRIAM, C. HART, Life and crop zones of the United States. *Bull. 10. Div. Biol. Survey.* 1898.

³ LIVINGSTON, B. E., A single index to represent both moisture and temperature conditions as related to plants. *Physiol. Res.* 1:421-440. 1916.

as specially favorable to wild berries, currants, blueberries, blackberries, and cranberries; and in more favorable places suitable for Irish potatoes, turnips, beets, the more hardy apples, and cereals such as wheat, oats, barley, rye, buckwheat, and also timothy.

From the phytogeographic standpoint, the Douglas Lake region is located in the transition zone between the northeastern coniferous forest province and the central or deciduous forest province. While the region is near the northern part of the transition belt, conditions are becoming more favorable to the deciduous forest province.

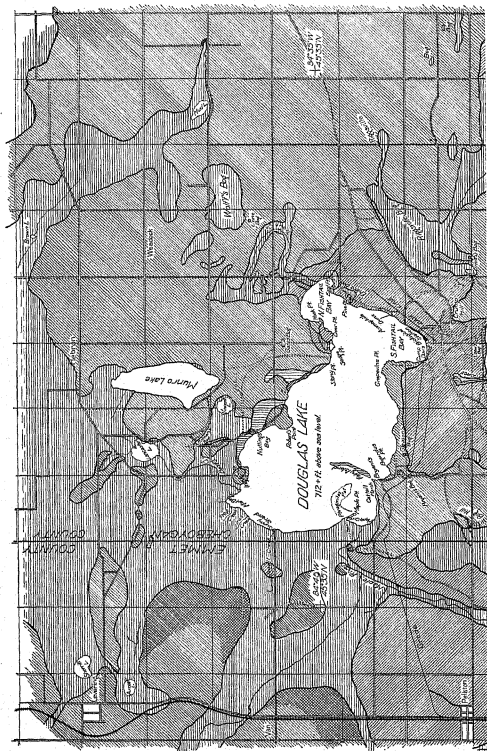
Factors

The Douglas Lake region is an area in which the climatic factors have been and are now favorable for the development of trees as a ground cover for the whole region. The temperature is moderate, the rainfall likewise is moderate, but amply distributed throughout the year. Snow is usually abundant, and remains on the ground for a long time.⁴ The soil is prevailingly of a sandy type. This may be mixed with gravelly or clayey material in the glacial moraines, but is nowhere a heavy loamy soil. In the lower parts of the region where bogs have developed the soil is largely of organic nature. Fire is probably the most important single factor in the region at the present time. It has operated extensively and repeatedly in the region since lumbering.

History of vegetation

Following the withdrawal of the ice, the different parts of the area became vegetated until, at the time of lumbering in the 1870's, three conspicuous types of vegetation were present. The poorer or sandy uplands were covered with pine forest (*Pinus Strobus* and *P. resinosa*), grading rather sharply into beech-maple forest on the better soils of the uplands. The lowlands were cedar bogs (*Thuja occidentalis*). Depressions which filled with water became lakes, and aquatic vegetation was developed in and bordering them. The region was predominantly one of extensive forest.

⁴ For the detailed figures of meteorology of the Biological Station see GATES, FRANK C., Meteorological data, Douglas Lake, Michigan. Papers of the Mich. Acad. of Science, Arts and Letters, 4: 475-489. 1924, and other papers referred to there.



With the lumbermen came the removal of the forest, first from the pineland, and later, even up to the present time, the beech-maple forest (*Fagus grandifolia*-*Acer saccharum*). Areas that were

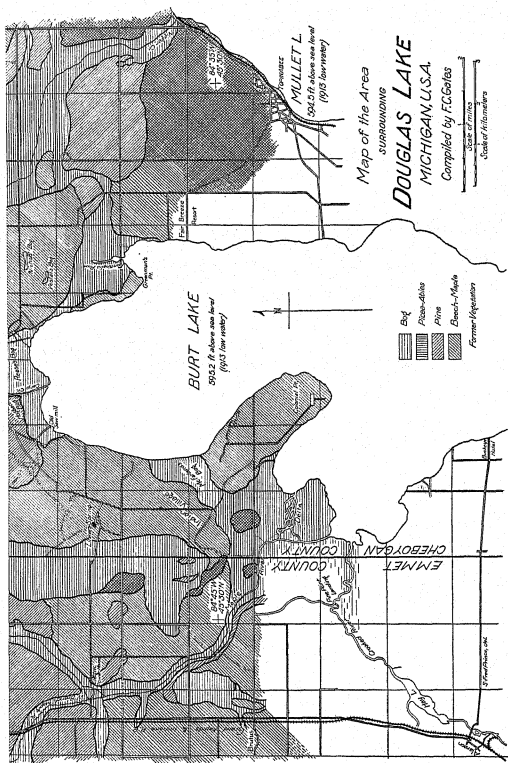


FIG. 1.—Map of area surrounding Douglas Lake, Michigan, showing approximate distribution of principal types of vegetation previous to era of lumbering.

lumbered were nearly always burned and usually burned repeatedly. The cedar bogs were largely cut and more or less burned, but, as bog conditions are much less favorable for extensive fires, such areas

are not so badly damaged. The immediate result of the clearing and burning was the installation of a new vegetation cycle over widespread areas. Fireweeds (especially *Epilobium angustifolium*) came first, and later shrubs and trees. Areas that have been repeatedly burned are now largely covered with aspens (*Populus* spp.). This type of vegetation is favored by the occasional fires, at the expense of the pines, beech, or maples.

Description of region

At the present time the region includes lakes and ponds in the depressions in which water accumulates, with rivers or smaller streams connecting them. In each there are appropriate types of aquatic vegetation, showing various stages of development from the deep water to the shore. The shore conditions around the larger lakes are those of the strand, and are subject to the action of storm waves and ice. Appropriate vegetation is very scanty because of the thoroughness of the ice work, and the fact that replenishment of suitable plants is not possible from the immediately surrounding vegetation, which is present under sharply different conditions. On a few of the most exposed shores, where opportunity is afforded for sufficient wind action in the right direction, small dunes are present behind the strand. Where shores are relatively protected, as in the smaller lakes or ponds, or on the lee sides or in coves in the case of the larger lakes, marsh development usually replaces that of strand, and leads rather abruptly into the distinctly land type of vegetation. Occasional seasons of high water are accompanied by such severe ice work that the region is not favorable for the development of low-land woods or bogs down into the water. River banks vary in vegetation, depending upon whether the bank is steep or low, and upon the character of the stream, whether swift or slow and whether subject to much ice work or not, and whether subject to human interference. Steep banks are likely to have a characteristic vegetation and different from the immediately adjacent area. Low banks where the streams are slow may not differ much from the adjacent area, except when there is a very radical change of soil type or some other local condition.

Land subject to overflow or spring flooding is vegetated with

lowland forest or in stages leading up to that stage, as willows, dogwoods, and similar thicket plants.

Rock shores and rock ledges are not present in the region close to Douglas Lake. Those washed over by Carp Creek in its descent into Lake Michigan at Cecil have a vegetation but little different from stream bottoms of other types.

On the lowland surrounding or adjacent to several of the lakes we find the cedar bog forest in considerable quantity, or stages of development which under normal conditions will sooner or later lead up to it, as for example, *Chamaedaphne*, *Carex lasiocarpa*, and *Larix laricina* associations. On the better land, in which there is more or less clay mixed with the sand which prevails in the region, we find a few virgin beech-maple forests, also several other forests in which selective cutting has taken place, leaving the poorer and smaller trees and modifying the ground conditions materially with the admittance of a great deal of light to the soil. There are many similar areas in which more or less burning has taken place and the vegetation in consequence represents various stages and mixtures of aspens and hardwoods, tending toward replacement of the beech-maple forest; also there are many areas in which the burning has been so severe that aspen dominance is complete and it is rather problematical whether the beech-maple forest will come back; and finally there are many areas in which the forest has been completely removed and the land used for agricultural or grazing purposes, in which case there is no chance of the return to the beech-maple forest, so long as such conditions are maintained. On the poorer soils of the uplands, which are only too evidently little but sand, one no longer finds any original pine forest, but finds many and extensive areas entirely dominated by aspens in various stages of development, depending upon the time of the last fire in that particular area or part of an area; also there is a number of areas in which seeding of pines is taking place relatively rapidly and several to many young pine trees are to be found among the aspens, with every indication of sooner or later replacing them entirely in the vegetation. There are a few areas in which the replacement has taken place and where we now have a young pine forest with trees 6-9 m. high and 20-30 years of age in areas somewhat protected from burning by their loca-

tion with respect to ridges or roads; and there are many areas in which aspen dies only to be replaced by aspen, as previous fires have completely destroyed any seeding pine trees and the nearest such are somewhat too far away even for stray seeding.

Cultivation

There is no longer any land used for agricultural purposes in the close proximity of the Biological Station, although one can find such at the edge of the region. Areas grazed by domestic animals are not sufficiently numerous to be studied satisfactorily. Actual farming is conducted on a small scale in a few places in the region. It consists largely of truck gardening for the few families and for the resorts of the region, together with some dairy farming. The horticultural possibilities of the area are slight, and are most likely to consist of apples and cherries. Small berries are quite abundant, but usually the supply is obtained from natural growth and not by cultivation. Such include particularly blueberries, strawberries (also the cultivated kinds), red raspberries, blackberries, dewberries, and cranberries. Good pasture land close to the Station is rather rare, but adjacent pasture land in very good years is excellent.

Associations

Forty-three associations may be recognized in the area. In their delimitation special attention has been given to the floristic composition, but in some cases, notably on the dunes, the physical factors of the habitat are so important that they need to be taken into active consideration. In other cases certain plant groups act apparently independently of the habitat and are found in places where they would not normally be expected, indicating that one cannot lose sight of the plants themselves, even though a classification based on habitat alone is much easier to construct.

In the beginning there was no such thing as an association. As disseminules get into a new area and develop, plants appear here and there, depending absolutely upon the growth of these disseminules, although the exact location may be a matter of the operation of one or more physical or chemical factors. As such plants usually grow separately and without keen competition, the result is a very open

assemblage. As these grow more closely together by becoming larger, by vegetative propagation, or by additional individuals coming into the interstices, the conditions become different. In the first place the crowding leads to the elimination of plants that cannot withstand such conditions, whether from lack of food or light, or because of the soil or other conditions. This results in the elimination of the plants that are unsuited and the spread of the plants that are there and are suited. The whole result is the condition which we call an association; in other words, a group of plants that live together under the conditions present.

In most cases the life of an association is interfered with by the invasion of disseminules from another association. If these do not find conditions suitable for growth, there is no possibility of succession. On the other hand, if conditions are favorable for the development of these invading disseminules but the plants do not crowd out the plants already there, there is merely an addition to the association. Such additions may merely add complexity to the vegetation, or they may serve as nuclei around which successions may take place under more favorable conditions. When the disseminules find suitable places for growth and development, however, and become plants which in one way or another dominate over the plants that were there, succession has taken place.

Succession

The process of succession is a natural one in which there are many contributing factors. The actual cause is and must of necessity be invasion and growth of a new order of plants in an area previously occupied by a group of plants. There may be a number of contributing or limiting circumstances, however, which many authors refer to as actual causes. The contributing circumstances which favor succession that are particularly active in this region are changes of water level, both permanent and fluctuating, formation of beach pools, flooding, ditching and cultivation, lumbering, changes in the reaction of the water, changes in the evaporating power of the air, running water, erosion, humification of the soil, and the work of certain insects and changes of light. Most prominent among the factors tending to prevent succession, or to check it

after it is started, are fire and the work of water in the form of streams, waves of lakes, and when frozen as ice.

Genetic series

By genetic series is meant a series of associations related in a continuous succession. Under normal circumstances a genetic series should begin with its lowest, and consequently ecologically simplest associations, and proceed through ecologically more complex associations until the climax for the given conditions is reached.

The duration of any genetic series depends entirely upon the duration of suitable conditions. In extreme cases a series may be completed within a year or two. Other series have been running for ages. It is possible for one series to change to another if conditions favor such a change and the necessary plants occur. Physiographic changes in the land frequently favor changes from one genetic series to another, as may easily be shown in the change of stream courses. Each series is usually a physiographic or biotic unit in the whole series of successions. It is separated from the whole series because of some striking peculiarities which make it distinct and consequently convenient to handle. A group of series fits into a complete discussion of successional relationships of the associations of any region.

In taking up genetic series three main groups can be recognized, two of which, designated as xerarch and hydrarch, are natural series depending upon the water content of the soil, and might together be designed as feralarch^s series. The third group (hemerarch) contrasts in being brought about by cultivation. Taking up first the hydrarch series, fig. 2C shows the associations that are normally found in the change from lake to dry land. The line running from lake to dry land is the condition of open lake with waves where plants are not present. The upper part of the figure shows the condition of the lake in relatively waveless parts, free from the severest ice work except at the very shore itself. The lower part of the figure brings out the condition in waveless parts of the lake, where there is continuous vegetation from the lake into the dry land associations. The situation in ponds and pools is similar to this last case.

^s GATES, F. C., Hemerarch and feralarch, two additional terms in ecology. *Science* 61:260. 1925.

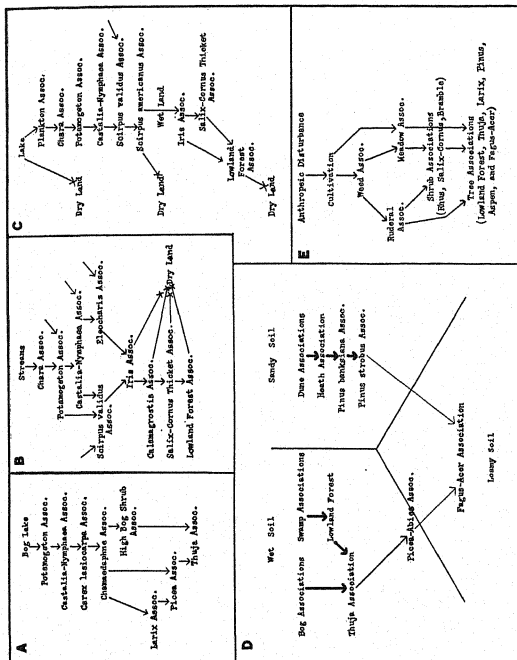


FIG. 2.—Diagrams showing usual successional relationships between associations of most important genetic series in region of Douglas Lake, Michigan: A, hydrarch series in bog lakes; B, hydrarch series in streams (X in place of arrowhead indicates juxtaposition); C, hydrarch series in the lakes; D, land series (successions indicated by light arrows are rare and involve profound changes of environment); E, relationships involved in return from hemerarch to feralarch series.

Lakes which form in relatively undrained areas show a different type of succession, which is exhibited in fig. 2 *A*. The succession proceeds from open water associations, through a mat developing over the water, and then into ericaceous shrubs and finally coniferous trees. The conditions in eroding streams are shown in fig. 2 *B*. The inception arrows here indicate that the series may begin in several places, and the crosses at the end of lines in place of arrows indicate that there may be an abrupt junction of the aquatic vegetation with that of the dry land, as for instance in the case of a bluff.

Land groups

Among the land groups there are three distinct conditions, which are collectively shown in fig. 2 *D*. The starting point from sandy soil is indicated in the upper right. In the upper left is shown the transition from lowland to upland. In this part of the figure the lowland forest and the *Thuja* association are given as the end members of wet ground genetic series. These associations may persist through the change from wet ground to conditions which partake very largely of upland, but whose vegetation has not yet had time to change to a true upland type; or they may persist in places where succession is impossible due to occasional flooding which continues to kill out such members of the upland association as have started during dry periods. In the lower part of the figure is shown the loamy soil on which the beech-maple association, which is the regional climax, represents the intrusion of the central deciduous forest province into the region. At present this is the one association of that province well represented in the region. The lines separating these groups indicate that before a change is made from one genetic series to another, a conspicuous change must be made in the habitat. For instance, the progressive drying, due to lower water tables or to the blowing in or washing in of material into low wet ground, builds it up sufficiently for the beech-maple forest to enter. Likewise the humification of sandy soil gradually permits the invasion of the beech-maple forest. The upland has been disturbed by lumbering, slashing, fire, and cultivation.

Summing up these types of disturbances in fig. 2 *E*, the general relationships are indicated by the interrelation of the types of associations that may develop there.

Having taken up the usual series from the conspicuous stand-points that are present in the region, for those who are particularly interested in the summation of the matter in a single diagram, fig. 3 is

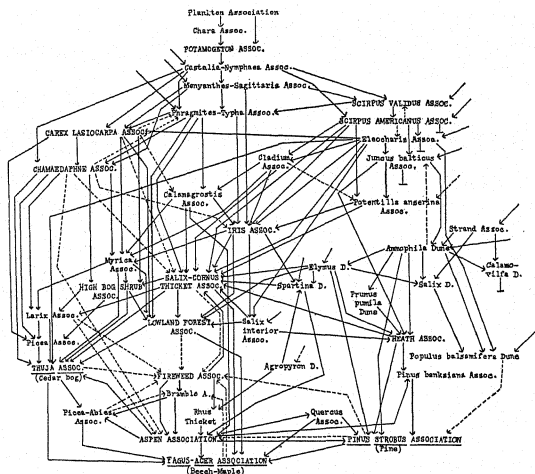


FIG. 3.—Diagram showing successional relationships exhibited between plant associations in region of Douglas Lake, Michigan: broken lines indicate secondary successions; solid lines, primary successions; an arrowhead, direction of succession, a cross line at end, that succession usually terminates here; an arrow from the open means that the association may start *de novo*; capital letters denote more important associations, while those underlined dominate the three principal soil types of region.

given. All facts with regard to succession between associations that have been observed in the region are here indicated. As in the other figures, the arrows point to the direction of succession; arrows coming from no association indicate the possibility of inception of a series; dotted lines represent the secondary associations following various disturbances. The three conspicuous soil types are indicated by underlining their climax associations. In interpreting a diagram of this sort, of course one must be careful to avoid the assumption

that every bit of ground in the region will go or has gone through all steps indicated here, as there are many cases where in given spots a complete series is never present, but steps may be jumped. For instance, the *Chamaedaphne* association may go to any one of eight different associations, although the usual case is indicated in the earlier diagram (fig. 2 A). As is readily seen in this figure, the vegetation of the region is very intricate, due to the great disturbances that have gone on in the past and which are far from being stabilized at the present time.

Summary

1. The Douglas Lake region is located in the northern part of the overlapping zone between the Northeastern Coniferous Forest Province and the Central Deciduous Forest Province in the extreme northern part of the Lower Peninsula of Michigan.

2. The former distribution of the most widely distributed plant associations is presented in a map of the region.

3. The intricate successional relationships between the plant associations which exist today are shown in six diagrams.

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TEMPERATURE AND RESPIRATORY ENZYMES OF APPLES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 352

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(WITH PLATE XI)

The recent interest in the marketing of food products has called attention to the lack of understanding of life processes that go on in these products. The early physiological reports on this group of plant structures are fragmentary and need revising, using modern methods. This is especially true in enzyme activity and the localization of elements, products, or processes.

THATCHER (15) carried out tests on diastase, invertase, tannase, esterase, protease, oxidase, and emulsin type enzymes in apples. He concluded that ripening changes were chiefly due to oxidases. OVERHOLSER and CRUESS (13) studied the oxidation system in Yellow Newtown apples in relation to the browning of apple tissue. They were able to separate an enzyme (peroxidase), an organic peroxide, and a tannin, using qualitative methods. Qualitative tests (using benzidine and peroxide) indicate that peroxidase is a prominent enzyme in apple tissue.

Respiration is one of the essential processes of living organisms. Early studies of this process, in the case of apples, were concerned chiefly with the respiration quotient. GERBER (5) made such a study, sealing the fruit in chambers. GORE (6) and MORSE (11) reported that respiration in apples followed Van't Hoff's rule, that is, it increased 2.9 and 2.38 times in these tests, with an increase in temperature of 10° C. Their tests were for short intervals, and include comparatively few measurements of respiration rates. KIDD and WEST (9) found that high carbon dioxide concentrations in the storage air injured the flavor of apples. They concluded that the gas supply surrounding the cells in apple tissue affects respiration products and the development of flavor. BURROUGHS (3) made very complete determinations of respiration rates in apples for a short

period following picking. He worked with a number of the winter varieties, Ben Davis, Baldwin, Wagener, Spy, and Wealthy. His records show an increase in respiration following picking with fruit held at 68.5° F. Early picked fruit had a smaller initial respiration rate than those picked later.

Methods

The work cited suggested a study of respiration rate from the time the fruit was picked until decay set in, with the hope of obtaining a general idea of the rate of life processes in ripening apples. The investigation was conducted during the summers of 1921 and 1922. A series of determinations was made on the summer varieties, Maiden Blush and Oldenburg, using two temperatures, 25° and about 0° C. A constant temperature was secured at 25° by using a Freas water thermostat, and at 0° by the use of a temperature box cooled by brine and electrically controlled. Both fruit and apparatus were inclosed in the cold box to reduce variations, but the temperature varied about 2°. The water bath did not vary more than 0.25°.

The fruit was secured from orchards in Michigan and northern Illinois. It was picked from a single tree in each case, carried to the laboratory, and placed under experimental conditions as soon as possible. The first determinations were made within twelve hours after picking for Maiden Blush samples, and within forty-two hours for the Oldenburg. All samples were run in duplicate. Each sample contained 1-3 kg. of fruit, and was kept constantly ventilated throughout the experiment.

Respiration rate was determined by measuring carbon dioxide produced, using the method described by GORE (6) as modified by KÜSTER (10). A standard solution of barium hydrate in two Reizet absorption towers was used to take up the carbon dioxide. Then the excess alkali was titrated against a standard solution of oxalic acid, using phenolphthalein as indicator. At the higher temperature, readings of carbon dioxide production were made daily, and at the lower temperature every day or two. Tables I and III show the amount of respiration for Maiden Blush and Oldenburg apples held at 25°, and tables II and IV present data on respiration of the same varieties at 0° C.

In every case the fruit was placed under experimental conditions as soon as possible after picking. A similar series of determinations was made for Winesap apples purchased on the market. This fruit

TABLE I
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 25° C.
MAIDEN BLUSH APPLE

TIME AFTER PICKING IN DAYS	SAMPLE <i>a</i>	SAMPLE <i>b</i>	AVERAGE
1.....	78.76	85.80	82.28
2.....	195.80	175.56	185.68
3.....	193.16	169.62	181.39
4.....	257.62	208.56	233.09
5.....	343.86	233.20	288.53
6.....	310.64	249.48	280.06
7.....	292.60	269.50	281.05
8.....	297.44	355.74	326.59
9.....	448.80	338.80	393.80
10.....	297.66	229.90	263.78
11.....	291.06	Decay started	
12.....	224.18		
13.....	221.54		
14.....	193.16		
15.....	191.62		
16.....	171.60		
	Decay started		

TABLE II
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 0° C.
MAIDEN BLUSH APPLE

TIME AFTER PICKING IN DAYS	SAMPLE <i>c</i>	SAMPLE <i>d</i>	AVERAGE
2.....	24.20	31.24	27.72
4.....	21.34	46.20	67.54
6.....	42.90	50.82	46.86
8.....	40.48	Discontinued	
10.....	7.48		
12.....	14.52		
14.....	20.90		
16.....	46.64		

had been in cold storage and was on display at the time of purchase (July 3). Samples were taken and handled as described for the Maiden Blush and Oldenburg apples. The data in tables V and VI indicate the rate of respiration in this fruit at 25° and 0° C. respectively.

Most apples are more or less bruised in harvesting and handling. Some determinations were made to discover whether bruising modified the carbon dioxide output. Specimens of Oldenburg apples were

TABLE III
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 25° C.
OLDENBURG APPLE

TIME AFTER PICKING IN DAYS	SAMPLE <i>a</i>	SAMPLE <i>b</i>	AVERAGE
2.....	55.88	47.52	51.70
3.....	75.02	75.46	75.24
4.....	59.84	68.20	64.02
5.....	60.94	68.86	64.90
6.....	62.26	63.14	62.70
7.....	54.56	57.20	55.88
8.....	48.62	58.96	53.68
9.....	53.90	53.90	53.90
10.....	52.14	50.38	51.26
11.....	53.02	52.80	52.80
12.....	37.62
13.....	46.86	45.76	46.20
14.....	37.18	35.42	36.30
15.....	40.48	43.34	41.14
16.....	41.36	38.72	40.04
17.....	22.66	41.58	32.12

TABLE IV
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 0° C.
OLDENBURG APPLE

TIME AFTER PICKING IN DAYS	SAMPLE <i>c</i>	SAMPLE <i>d</i>	AVERAGE
3.....	151.58	210.98	181.28
5.....	29.26	39.38	34.32
7.....	33.22	45.32	39.27
9.....	39.82	47.30	43.51
11.....	66.88	70.18	68.53
13.....	89.98	29.04	59.51
15.....	29.26	33.00	31.13
17.....	36.74	25.96	31.35

bruised about half way through the apple, but without visibly breaking the skin. Such severe bruising should give a maximum response, but without complications arising from increased accessibility of oxygen. They were tested only at 25° C. The results are shown in table VII.

TABLE V
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 25° C.
WINESAP APPLE

TIME AFTER PURCHASE IN DAYS	SAMPLE a	SAMPLE b	AVERAGE
1.....	35.42	38.72	37.07
2.....	29.92	29.04	29.48
3.....	23.54	21.56	22.55
4.....	24.20	21.56	22.88
5.....	23.98	22.00	22.99
6.....	19.58		
7.....	20.46	19.80	20.13
8.....	21.34	22.66	22.00
9.....	24.86	21.56	23.21
10.....	22.66	26.84	24.75
11.....	28.82	21.56	25.19
12.....	27.50	18.48	22.99
13.....	24.86	21.12	22.99
14.....	25.08	17.38	21.23
15.....	19.14	17.38	18.26
16.....	23.10	25.96	24.53
17.....	19.36	22.66	21.01
18.....	22.00	22.22	22.11
19.....	16.50	25.52	21.01
20.....	20.46	23.32	21.89
21.....	19.36	25.96	22.66
22.....	18.48	24.20	21.34
23.....	17.16	19.36	18.26
24.....	19.36	23.76	21.56
25.....	17.82	18.48	18.15
26.....	18.70	25.52	22.11
27.....	18.26	18.26	18.26

TABLE VI
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 0° C.
WINESAP APPLE

TIME AFTER PURCHASE IN DAYS	SAMPLE c	SAMPLE d	AVERAGE
1.....	56.98	15.28	36.13
3.....	26.84	17.82	22.33
5.....	12.54	14.30	13.42
7.....	18.48	14.30	16.39
9.....	16.50	25.96	21.23
11.....	20.46	26.40	23.43
13.....	15.40	24.20	19.80
15.....	29.48	15.18	22.33
17.....	22.22	22.22	22.22
19.....	25.08	20.68	22.83
21.....	25.52	29.92	22.72
23.....	9.90	14.96	12.43
25.....	12.76	12.10	12.43
27.....	32.12	16.72	24.42

TABLE VII
MILLIGRAMS OF CO₂ PER KILOGRAM HOUR AT 25° C.
OLDENBURG APPLE BRUISED

TIME AFTER BRUISING IN DAYS	SAMPLE <i>a</i>	SAMPLE <i>b</i>	AVERAGE	CHECK AVERAGE
1.....	72.16	60.06	66.11	51.70
2.....	60.72	60.50	60.61	75.24
3.....	63.36	63.36	63.36	64.02
4.....	64.68	62.04	63.36	64.90
5.....	69.96	57.64	63.80	62.70
6.....	69.30	64.46	66.88	55.88
7.....	70.62	60.06	65.34	53.68
8.....	60.06	52.58	56.32	53.90
9.....	70.84	48.40	59.12	51.26

Respiration determinations

The outstanding fact revealed by the data presented is the difference in respiratory behavior of Maiden Blush and Oldenburg apples, with respect to temperature changes. The rate for Oldenburg varied little with the temperature, while that for Maiden Blush showed a very large depression of respiration at 0° C. It should be noted that catalase activity is also low in the Oldenburg apple. The Maiden Blush specimens were somewhat immature, which may partially account for the low initial rate. Bruising had little effect on respiration, although it hastened decay. Unless injury is accompanied by greater freedom of gaseous exchange, it seems to exert only a minor influence upon the respiration rate. This result is similar to that secured by JOHNSTONE (7, 8) with the sweet potato. Maiden Blush mellows rapidly, while Oldenburg is a remarkable keeper for a summer apple. This would be expected in view of the respiration behavior here reported. The over-ripe specimens of Winesap gave little variation in rate due to temperature.

ENZYMES

CATALASE.—Oxidase and catalase activities are often reported as being correlated with respiration. It was decided, therefore, to make some determinations of the amount and local distribution of these enzymes in the fruit. Some idea of the localization of the enzymes is necessary in order to take samples intelligently. In the preliminary work catalase determinations varied greatly, depending

on the amount of skin and subepidermal tissue occurring in the sample. To obviate this variability, all samples for catalase determination were taken with a cork borer, going in through a cheek to the core of the apple. Precipitated calcium carbonate was used to neutralize the acidity of the tissue, and the dry weight was obtained from a duplicate sample taken from the same apple at the same time and about the same size as that used in the determination. Accuracy of the catalase determination requires the grinding up of all the skin.

TABLE VIII

LOCALIZATION OF CATALASE IN FRESH OLDENBURG APPLES; CC. OF O_2
LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SKIN AND SUBEPIDERMAL TISSUE	FIRST CM. UNDER SKIN	SECOND CM. UNDER SKIN	CARPEL REGION
1	13.95	0.00	5.40	4.05
2	29.80	4.50	6.20	6.75
3	36.90	6.75	8.10	8.10
5	47.67	7.65	10.35	8.55

TABLE IX

CATALASE ACTIVITY IN FRESH OLDENBURG APPLES; CC. OF O_2
LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE <i>a</i>	SAMPLE <i>b</i>	AVERAGE
1	0.54	0.51	0.52
3	1.65	1.25	1.45
5	2.59	1.73	2.16
10	3.30	2.33	2.81

Hydrogen peroxide was used from a single fresh bottle secured from the Oakland Chemical Company, and all gas readings were reduced to 760 mm. pressure and 0° C. The apparatus used was a modification of that described by APPLEMAN (1). All determinations are for the equivalent of 0.5 gm. samples dry weight.

Tables VIII–XI present the results of catalase determinations. Table VIII shows the local distribution of catalase activity in an Oldenburg apple, while tables IX and XI provide a comparison of catalase in representative samples of Oldenburg and Winesap. Tables X and XI present the data for fruit (Winesap) stored at 0° and 25° C. respectively.

Catalase is much more active in the periphery of the apple (Oldenburg), and it is evident that the Oldenburg is low in catalase activity as compared with Winesap. This difference is not correlated with their respiration rates, for the Oldenburgs at 25° C. respired just about twice as rapidly as the Winesaps at the same temperature. Holding Winesaps at 25° C. brings about an increase in their catalase activity over those held at 0° C., as is seen from the data of tables X and XI. The significance of this difference is not known, since

TABLE X

CATALASE ACTIVITY IN FRESH WINESAP APPLES HELD AT 0° C.; CC. OF O₂
LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE a	SAMPLE b	SAMPLE c	SAMPLE d	AVERAGE
1.....	4.56	6.43	5.75	6.12	5.71
3.....	10.86	11.21	12.45	13.48	11.97
5.....	15.07	16.90	16.87	18.18	16.75
10.....	21.02	24.57	22.74	25.53	23.46

TABLE XI

CATALASE ACTIVITY IN FRESH WINESAP APPLES HELD AT 25° C.; CC. OF O₂
LIBERATED BY FRESH EQUIVALENT 0.5 GM. DRY SAMPLE

TIME IN MINUTES	SAMPLE a	SAMPLE b	SAMPLE c	SAMPLE d	AVERAGE
1.....	8.21	8.19	10.68	7.56	8.66
3.....	19.17	18.60	22.18	16.80	19.18
5.....	26.45	25.50	29.40	23.23	26.14
10.....	37.38	35.98	38.80	33.18	36.33

the respiration rates for this apple at 0° and 25° C. are practically the same.

OXIDASE.—Oxidase determinations, both qualitative and quantitative, were made in 1923 (4) and 1925. The simplified BUNZEL (2) apparatus, with pyrogallol or pyrocatechin as an absorbent, was used for the quantitative determinations. Color determinations, using an alcoholic solution of benzidine, as described by TUNMANN (16), were made for over fifty varieties of apples and a few samples of other pomaceous fruits. Many of these color determinations have been preserved in the form of photographic records. Tables XII and XIII show the quantitative distribution of oxidase in Winesap

and Yellow Transparent apples, and figs. 1 and 2 show the difference in local distribution of oxidase in these varieties.

Both the color and quantitative determinations indicated decreased oxidase activity when the fruit was hard ripe. This period was also characterized by less respiration than for ripe fruit. Red Astrachan apples showed this depression very clearly in the color tests (fig. 3). The decrease in oxidase activity at the hard ripe stage was quite marked in the periphery tissue of Yellow Transparent, and less marked in the core tissue of the same specimen.

TABLE XII

LOCALIZATION OF OXIDASE ACTIVITY IN WINESAP APPLES; CC. OF O₂
ABSORBED BY 5 CC. OF JUICE IN ONE HOUR

TISSUE	SAMPLE a	SAMPLE b	SAMPLE c	AVERAGE
Periphery.....	Trace	0.020	0.075	0.032
Core.....	0.290	0.143	0.259	0.231

TABLE XIII

LOCALIZATION OF OXIDASE ACTIVITY IN YELLOW TRANSPARENT APPLES;
CC. OF OXYGEN ABSORBED BY 5 CC. OF JUICE IN ONE HOUR

TISSUE	IMMATURE	IMMATURE	HARD RIPE	NEARLY EATING RIPE	OVER-RIPE	AVERAGE
Periphery....	0.090	0.080	0.000	0.023	0.066	0.052
Core.....	0.154	0.080	0.046	0.161	0.110	0.110

Winesap apples purchased on the market in July showed a higher oxidase activity than fresh picked specimens of Yellow Transparent. It is quite possible that the amount of acidity per variety influences this determination. Preliminary tests on sweet apples (low in acidity) indicate this.

The localization tests are even more interesting than the amount of oxidase activity per variety. Many varieties tend to have oxidase activity localized near the core. Varieties like Maiden Blush often show this localization in the periphery of the fruit (fig. 4). This suggested that localization of oxidase activity might be influenced by gas exchange. If such an exchange took place through the core, it ought to be possible, in time, to influence oxidase activity by sealing the

calyx tube in the hard ripe stage. Specimens of McIntosh, Delicious, Baldwin, and Spy treated in this way gave a negative test for oxidase activity in the core region after about two months at living room temperatures.

SHAW (14) found that northern grown specimens of Ben Davis were more conical than southern. Long shaped, conical specimens of Ben Davis having considerable flesh between the core and calyx tube showed oxidase activity in the periphery of the fruit. This suggests a climatic influence on oxidase activity in varieties of apples.

LOCALIZATION OF IRON

Iron is essential for chlorophyll development, and may be directly connected with the respiratory process. WARBURG (17) considers

TABLE XIV
LOCALIZATION OF IRON IN WINESAP; PERCENTAGE OF
FRESH WEIGHT

TISSUE	SAMPLE <i>a</i>	SAMPLE <i>b</i>	AVERAGE
Periphery	0.00427	0.00592	0.00509
Flesh	0.00126	0.00152	0.00139

iron the only respiration catalyst of plant and animal cells. It was considered worth while, therefore, to secure some information on the localization of this element. Potassium ferrocyanide color tests were made on ashed samples of parts of the fruit. These qualitative tests indicated some localization of iron in the periphery.

Quantitative determinations for iron were then made on flesh and periphery samples. These were taken by scraping with a glass knife, and all determinations were made in duplicate. The blanks indicated a very slight trace of iron in the reagents. The Neumann method, titrating with sodium thiosulphate, was used. Table XIV presents the results of these determinations for the Winesap variety.

Iron and catalase activity, therefore, are localized in the same general region of the Winesap.

Summary

1. Apple varieties vary greatly in respiration rate, and under some conditions show very little temperature response.
2. Respiration determinations give very little indication of ripening changes among varieties of apples after picking.
3. Oldenburg and Winesap apples respire less rapidly than Maiden Blush, other things being equal.
4. Skin and subepidermal tissues have comparatively high catalase activity.
5. Respiration rate and catalase activity are not closely correlated among apple varieties.
6. Oldenburg apples have low catalase activity compared with Winesap, together with a higher respiration rate.
7. Oxidase activity tends to decrease in hard ripe apples (Yellow Transparent and Red Astrachan).
8. Many apple varieties show greater oxidase activity near the core. Such varieties normally have a gas exchange through the calyx tube and core.

The writer desires to acknowledge his indebtedness to Professor C. A. SHULL and Dr. S. V. EATON of the University of Chicago for their kindly interest and many helpful suggestions through the course of the experiment.

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EXPLANATION OF PLATE XI

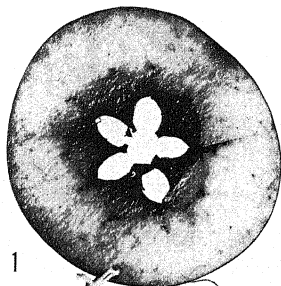
FIG. 1.—Oxidase activity in Winesap apples: dark color indicates oxidase activity; compare with table XII.

FIG. 2.—Benzidine oxidase activity test for Yellow Transparent apples; compare with table XIII.

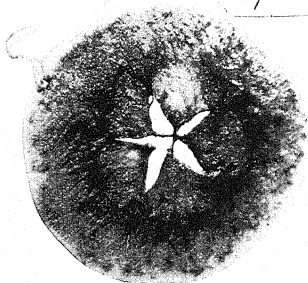
FIG. 3.—Hard ripe Red Astrachan apples giving very small test for oxidase ripe specimens show considerable oxidase activity throughout the apple.

FIG. 4.—Oxidase activity in Maiden Blush apples; Westfield and northern grown Ben Davis apples show similar localization of oxidase activity.

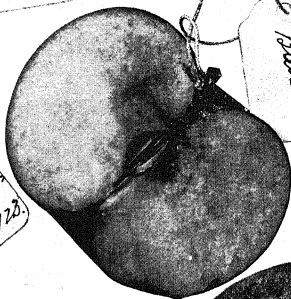
FIG. 5.—Specimen of Maiden Blush showing flesh between calyx tube and core cavities.



Winesap
4/18/23

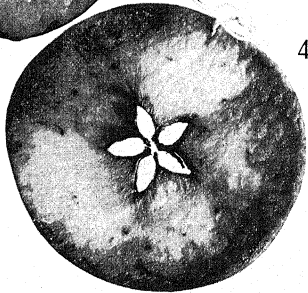
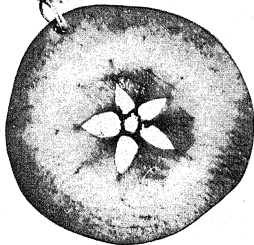


Yellow
Transparent
Aug 3, 1923

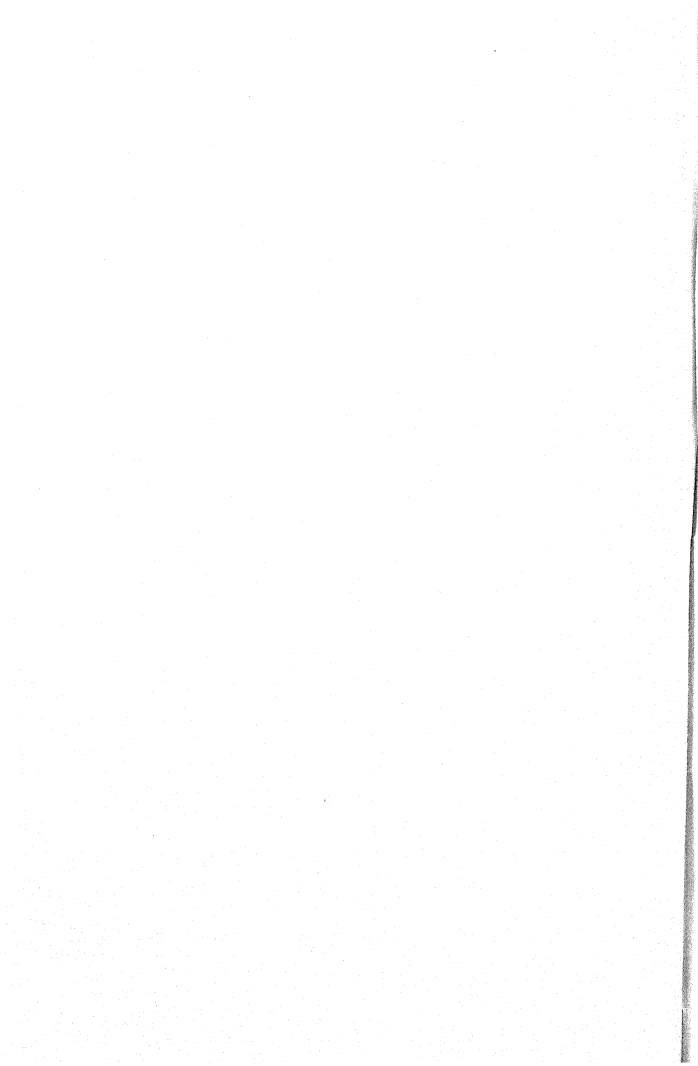


Maiden
Blush

R. Aethiops
immature
Aug 3, 1923



Maiden
Blush
10/17/23.



SURFACE FORCES OF SOILS WITHIN THE RANGE OF HYGROSCOPIC MOISTURE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 353

H. S. WOLFE

(WITH ONE FIGURE)

Introduction

The past decade has seen numerous and important advances in our knowledge of soil moisture, and in our recognition of the importance of the part played by the colloidal fraction of the soil in determining its moisture retaining properties. The conclusions which were reached by BOUYOUCOS (4, 5) as a result of the application to soil moisture problems of the dilatometer method employed with inorganic hydrogels by FOOTE and SAXTON (9, 10), have been particularly stimulating to research. His theory of the different physical states of the water present in the soil as soil moisture was at once challenged by various workers. KEEN (14), PARKER (16), and SHULL (21) have all examined this hypothesis, compared the data on which it was founded with their own data on a different angle of the problem, and concluded that there is no break or abrupt change in the physical condition of the soil moisture.

The fundamental error in the hypothesis of BOUYOUCOS seems to have been in assuming the trustworthiness of the dilatometer method. The work of VANZETTI (25) and LEHNER (15) has shown that this method does not give a reliable measure of the combined water in inorganic hydrogels, and consequently can hardly give an indication of the nature of the water present as soil moisture in soils, as PARKER has pointed out. THOMAS (23) has brought forward strong evidence that even with a relative humidity of only 1 per cent, the soil particles are covered with a film of water at least one molecule in thickness. The studies of VAN BEMMELEN (24), ZSIGMONDY (26), VANZETTI, and LEHNER on hydrogels; and of PATTEN and GALLAGHER (17), CAMERON and GALLAGHER (7), KEEN (13), SHULL (20), and PARKER on soils have all indicated that the water

present is all in the same physical state, but that it is held with great forces as its amount becomes small, forces so great, as PARKER indicates, that it cannot be frozen even at -78°C .

In his résumé of the problem of soil moisture classification, PARKER has discussed the four methods by which these forces may be estimated relative to different moisture contents, and has shown the striking similarity in the curves obtained by these different methods. They are: (1) rate of evaporation, (2) freezing point depression at different moisture contents, (3) vapor pressure during dehydration, and (4) equilibrium relations of the soil with seeds of known water absorbing power. Of these methods, the second and fourth are by far the more sensitive, he says, and give curves of greater accuracy; but only the last method gives direct estimations of the values of the forces operating.

The only work done with this soil-seed equilibrium method has been that by SHULL (20), who devised it. Preliminary investigations had seemed to show that the graph which he presented for Oswego silt loam indicated too high a value for the forces at the point of maximum curvature. In view of the importance of this method of determining the forces with which soil moisture is retained, and of the questionable accuracy of the only published curve, therefore, it has seemed wise to reinvestigate more carefully the portion of the curve lying within the range of hygroscopic moisture, as this region includes all the debatable portion of the curve.

Methods and materials

The method and even the same apparatus described previously (20) were employed in this research, with certain modifications as noted. The rotator apparatus has been described and illustrated, so that no further reference to it is necessary here. Instead, however, of attempting to bring soils to an arbitrary water content by mixing the dry soil with water, air-dry soils were exposed for various periods of time in saturation chambers of the type devised by HILGARD (12) for studying the hygroscopic coefficient of soils. Since at least 24 hours are required for even a very thin layer of soil to reach its approximate maximum hygroscopic moisture content, it was conceived that by exposing the soil in layers of uniform thickness for

periods of time increasing by hourly increments from one up to 30 hours, a series of hygroscopic moisture contents would be obtained covering the desired range and with no large gaps in the line. Accordingly this was the procedure followed.

The soil was transferred as rapidly as possible from the saturation chamber to the rotator bottle, a known weight of air-dry seeds of *Xanthium* added, the soil shaken thoroughly to distribute the seeds well throughout it, and the bottle put into the rotator for two weeks. At the end of this time the bottles were opened, a few grams of soil rapidly transferred to weighing bottles, the seeds brushed free of dirt and put into weighing bottles also, and both soil and seeds weighed as soon as possible. From the increase in the weight of the seeds, the equivalent osmotic pressure of the soil "back-pull" was determined by inspection of a curve constructed from data previously given (20). From these data the hygroscopic moisture of the soil in equilibrium with the seeds was determined by drying the soil sample to constant weight in a 105° C. oven, and expressing the loss in weight as percentage of the oven-dry weight. The two sets of values thus obtained (equivalent osmotic pressure in atmospheres and hygroscopic moisture percentage) were plotted against each other to make more evident their relationship.

The seeds employed were from the laboratory stock of *Xanthium pennsylvanicum* Wallr. fruit, gathered from moderately pure line plants grown in the garden. Only the lower seeds were used, in order that the results might be based on as few non-uniform factors as possible, although such experiments as have been done with the upper seeds indicate no difference in their behavior in this respect from the lower seeds.

Two heavy loam soils were selected, following preliminary trials of a number of different soils to determine which were best suited for further experimentation, because they were readily brushed free from the seeds and because they resembled the soil used previously in this work. One came from a garden in Homewood, Illinois, and is designated "garden loam," while the other was one of a series of soils used in laboratory work, and is called simply "heavy loam." The following physical and chemical properties of these soils were determined and are compared in table I with the same properties

of the Oswego loam used by SHULL. All these are percentages of the absolute dry weight. The wilting coefficients and the moisture equivalents were calculated from the hygroscopic coefficient in the

TABLE I

TYPE OF SOIL	PERCENT- AGE HYGRO- SCOPIC MOISTURE	PERCENT- AGE HYGRO- SCOPIC COEF- FICIENT	PERCENT- AGE WILTING COEF- FICIENT	PERCENT- AGE MOISTURE EQUIVA- LENT	PERCENT- AGE LOSS ON IGNITION
Oswego loam . . .	5.5	13.0	19.1	35.2
Garden loam . . .	2.6	11.0	16.2	29.6	9
Heavy loam . . .	3.9	14.0	20.6	37.8	11

TABLE II

HOURS IN SATURATION CHAMBER	HYGROSCOPIC MOISTURE OF SOIL IN PERCENTAGE OF OVEN-DRY WEIGHT		MOISTURE INTAKE OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT		EQUIVALENT OSMOTIC PRESSURE IN ATMOS- PHERES	
	Heavy loam	Garden loam	Heavy loam	Garden loam	Heavy loam	Garden loam
1	4.12	4.10	3.21	3.70	580	540
2	5.00	4.32	4.20	4.07	490	500
3	5.24	4.70	4.43	6.05	465	360
4	5.40	4.85	5.35	6.32	410	335
5	5.95	5.15	6.67	8.80	350	220
6	6.22	5.51	7.58	10.10	270	155
7	6.30	7.96	255
8	6.84	5.55	10.60	11.73	177	130
9	7.00	10.75	165
10	7.56	5.98	11.80	14.60	130	97
12	7.83	6.15	12.80	15.27	116	91
14	8.20	6.41	14.25	17.21	110	78
18	8.72	6.50	15.16	(20.97)	92	(61)
20	8.85	6.57	18.93	18.94	70	70
22	9.15	6.76	19.52	19.74	67	66
24	9.30	7.00	20.47	20.59	63	63
26	7.60	22.95	54
28	7.73	24.05	50
30	10.33	8.01	22.53	26.17	54	43
72	13.43	10.25	33.15	33.60	26	26

last two soils, while the process was reversed for the first one, the formulas of BRIGGS and SHANTZ (6) being employed.

Experimental results

LOAM SOILS AT VARIOUS MOISTURE CONTENTS.—The results of the experiments with heavy loam and garden loam are presented in

table II, while fig. 1 gives the curve obtained by plotting soil moisture against surface forces for these two soils and for that investigated previously.

The greatest care was taken to secure uniformity in the handling of the material, and to reduce sources of error to a minimum. ALWAY, KLINE, and McDOLLE (2) have pointed out the error due to

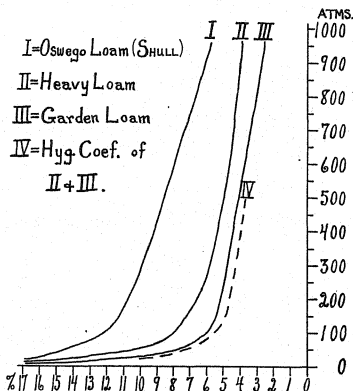


FIG. 1.—Curves showing relation of soil moisture content to soil surface forces: ordinates, surface forces of soil in atmospheres; abscissae for curves I, II, and III, moisture content of soil in percentage of absolute dry weight; for curve IV, moisture content of soil as percentage of hygroscopic coefficient (multiply figures by 10).

the loss of moisture while transferring soil from the saturation chamber to the weighing bottle, and compare the values for soils exposed during transfer for 30 and for 150 seconds. In these experiments the soil was exposed for less than 10 seconds. About 5 minutes were required for the cleaning of the seeds of each set, and they were partially exposed to the air during this period, but this has been found much too short a period to affect the resulting moisture content significantly.

Six samples of each soil constituted a series, the capacity of the rotator, and the same soil was air-dried for use in the next series. THOMAS (23) has pointed out that drying a soil previous to exposure increases its vapor pressure later for a given moisture content, but this is true only when the soil has been dried considerably below air-dry.

SOILS AT THE HYGROSCOPIC COEFFICIENT.—Several different soils were brought to the hygroscopic coefficient by three days' exposure in the saturation chamber. Table III presents the results obtained when the surface forces at this point were determined. For the

TABLE III

TYPE OF SOIL	MOISTURE IN- TAKE OF SEEDS IN PERCENTAGE OF AIR-DRY WEIGHT	EQUIVALENT OSMOTIC PRES- SURE IN ATMOSPHERES
Sandy loam.....	31	30
Silty loam.....	34	25
Heavy loam.....	33	27
Garden loam.....	33	27

sandy loam the value given is the average of three determinations, and for the other soils it is the average of two determinations.

Since there is a slight loss of moisture during the experiment, so that the soils at its close are a little below the hygroscopic coefficient when in equilibrium with the seeds, the osmotic pressure equivalents are slightly too large. At the hygroscopic coefficient the value becomes about 25 atmospheres, when the correction for the moisture loss is made. Since the important value is the moisture content at the close of the experiment, that is, that when the seeds are in equilibrium with the soil, it is manifestly immaterial that the soil should not have exactly the same moisture content at the close as at the beginning. The loss is not due to any leakage of the apparatus, but to the length of time that the soil must be exposed in the process of getting it into the rotator bottle, and to loss of moisture within the bottle to saturate or bring into equilibrium with it the air in the bottle. The part actually absorbed by the seeds is negligible.

Discussion

The nature of the forces at work in the establishment of equilibrium between seeds and soil has been elucidated by SHULL (20), so that it is not necessary to consider that phase of the problem here. These data, however, make it clear that the hygroscopic coefficient represents a rather definite equilibrium point in the system soil particle-water vapor, for in these soils of different hygroscopic coefficient the force with which the soil particles hold back water is very constantly about 25 atmospheres.

This has been suggested previously by SHULL (21) on the basis of unpublished data, and is in line with what he has shown (20) to be the case with regard to the wilting coefficient, at which point a wide variety of soils were all found to hold back moisture with a force of 3-4 atmospheres. Incidentally it may be noted that when the surface forces at the wilting coefficient are calculated for the soils used in this experiment by extrapolation of the curves, the values agree closely with the preceding. The Oswego loam was shown by SHULL as having a back-pull of about 75 atmospheres at its hygroscopic coefficient, however, if this point may be calculated by the BRIGGS-SHANTZ formula ($\text{hygroscopic coefficient} = \text{moisture equivalent} \times 0.37$). Using this equation the desired value is obtained as 13 per cent. Since neither SHULL nor the authorities he cites for the soil, CARTER and SMITH (8), make any mention of its hygroscopic coefficient, we cannot be sure that this value is correct, for ALWAY and RUSSELL (3) have shown that these formulas of BRIGGS and SHANTZ are not valid for all soils. They consider, however, that in general the mathematical derivation of the hygroscopic coefficient from an empirically determined moisture equivalent is more accurate than similar derivation from the wilting coefficient, and the figures furnished by BRIGGS for the Oswego loam (20) make it evident that he actually determined the moisture equivalent and derived the wilting coefficient from it, so that we are rather safe in deriving the hygroscopic coefficient similarly. It is felt that the abnormally high osmotic pressure equivalent found by SHULL for this soil at its hygroscopic coefficient is to be explained by his imperfect method for obtaining desired moisture contents. Soil mixed with water undoubtedly has its particles much less uniformly coated with

moisture films than when it is exposed in thin layers to a moisture laden atmosphere.

It has been noted that when the curves for all three of the soils in fig. 1 are prolonged to the point of the wilting coefficient, they all agree at about 4 atmospheres for the soil back-pull at this point. Now THOMAS (22) observed that the wilting point was reached when the vapor pressure of the soil had been lowered 0.4 mm. of mercury from its value at maximum air saturation. This he found to be equal to an osmotic pressure of 26 atmospheres in solution at 25° C., and so the soil should be retaining moisture with that force at the wilting coefficient. This is not at all in agreement with the numerous direct determinations, both of the actual force at the wilting coefficient, and of the moisture content when a force of 26 atmospheres is being exerted.

PURI, CROWTHER, and KEEN (18) call attention to the fact that at about 50 per cent relative humidity there is a minimum change in the moisture content of the soil for a given change in relative humidity. They suggest, therefore, that there may be thus derived a value which will express in a single soil constant as definite a characterization of the soil as any other single one. Air-dry soil has about 50 per cent relative humidity, and would thus be the basis of evaluation of the soil constant. In the present investigation the air-dry moisture content showed a much less constant relation to the surface forces than did the hygroscopic coefficient.

Very interesting results were obtained by comparing the hygroscopic moisture of the two soils used in this experiment, expressed as percentage of the hygroscopic coefficient, at certain definite osmotic pressure equivalents. From the remarkable uniformity of these values for these two soils, as shown in table IV, we are led to question whether the hygroscopic coefficient may not possess more significance as a soil constant than has lately been conceded to it.

Curve IV in fig. 1 shows graphically these relationships between the surface forces of the soil and its moisture content expressed in terms of the hygroscopic coefficient, and the striking similarity in its form to that of the usual soil moisture curves beside it. The force with which soil particles retain moisture appears to be a function of the hygroscopic coefficient, and is equal for different soils

when they contain the same percentage of their maximum hygroscopic moisture capacity.

In accordance with our modern concepts of the part played by colloidal matter in soil surface forces, we should expect that sand, which is deficient in colloid content, would fail to follow the same laws as soils, and so it proves. KEEN (13) found evaporation from sand to proceed quite differently from that from soil, following instead the usual laws of physical diffusion. SHULL (20) found coarse sand to give values of back-pull at the wilting coefficient considerably divergent from those uniformly obtained for the other seven

TABLE IV

OSMOTIC PRESSURE EQUIVALENT (ATMOSPHERES)	SOIL MOISTURE CONTENT AS PERCENTAGE OF HYGROSCOPIC COEFFICIENT	
	Heavy loam	Garden loam
25	100	100
50	73	71
75	62	60
100	57	55
150	51	50
200	47	46
250	45	45
500	36	38

different soils examined. The present experiments have shown that at the hygroscopic coefficient a coarse sand retains moisture with an equivalent osmotic pressure much below that for soils, and that there is no constant relationship between those forces and the hygroscopic coefficient. If this equilibrium point may be calculated for the sand used in the present work, it was there found to correspond to a surface force of only 10 atmospheres, and for different sands this value differs also. We are dealing evidently with quite different factors from those which operate in soils. It is of some interest to note that both in the present and in previous work, sand at about three times its air-dry moisture content showed about 30 atmospheres of surface force, but in the former case this was at 10 per cent of the hygroscopic coefficient, and in the latter case at about 50 per cent of that moisture content.

The application of this determination of the soil surface forces at the hygroscopic coefficient may be made through the work of ALWAY (1), who noted that many desert perennials could live in soils which were below this moisture content. This means that they were able to extract water from the soils against a back-pull of more than 25 atmospheres of force. Desert plants are well able to produce within their root cells osmotic forces far greater than this, as HARRIS, LAWRENCE and GORTNER (11) have demonstrated, and, furthermore, are able to withhold water from transpiration by the development in their leaves of very powerful hydrophile colloid pentosans and mucilages, as various workers at the Desert Laboratory have pointed out (21).

A number of improvements in technique have been suggested by various workers since this work was begun, and more accurate methods are now available which should give even more certain data. ROBINSON (19) has pointed out the advantages of 2 per cent H_2SO_4 for use in obtaining the hygroscopic coefficient, since it gives values approaching those obtained over water, and yet permits the establishment of a real equilibrium such as is never obtainable with HILGARD's method. THOMAS and PURI, CROWTHER and KEEN have added greatly to our knowledge of methods for soil vapor pressure determination. They have established the value of the vacuum desiccator, using concentrated H_2SO_4 as dehydrating agent, for the drying of soils to constant weight, and of the same apparatus with H_2SO_4 - H_2O mixtures of known vapor pressure for producing varied soil moisture contents. Drying over H_2SO_4 gives more constant and lower absolute dry weights than does drying in the 105° C. oven, and avoids the changes in colloidal structure incident to heating. The difficulties due to the hysteresis of the colloidal portion of the soil in its water relationships are just as real with air-dry soil as with soil saturated with moisture vapor. The value of the constant suggested by KEEN and his co-workers lies in the fact that moisture content varies little over a considerable range of relative humidity of about 50 per cent relative humidity, and so a definite soil moisture content is readily obtained with some certainty. But with their method of producing desired relative humidities, it is possible to obtain one of 95 per cent, which is about what can be obtained in the usual HILGARD method, with equal precision, and to produce very uniform

hygroscopic coefficients. Until the moisture content at 50 per cent relative humidity is shown to have more constant relation to the empirically determined soil surfaces, as further work may show it to have, the hygroscopic coefficient remains our most useful concept as a soil characterizer.

Finally it may be noted briefly that the smoothness of the curves given in fig. 1 for the soil surface forces indicates once more the impossibility of considering that water is present in more than one physical state in soils normally.

Summary

1. Various soils are shown to hold moisture when at their hygroscopic coefficient with a force uniformly equivalent to about 25 atmospheres, as measured by the seed-soil equilibrium method of SHULL.

2. For at least the two loam soils investigated, the surface soil forces within the range of hygroscopic moisture are shown to be constant for any given soil moisture content, expressed as percentage of the maximum hygroscopic moisture capacity of the soil, that is, to be functions of the hygroscopic coefficient.

3. Sand does not follow the same laws in regard to moisture holding forces as do soils.

4. The curve for soil forces given previously is shown to indicate too high values for the force with which water is retained through that portion of the hygroscopic moisture range near saturation with water vapor.

The writer desires to express his thanks to Professor CHARLES A. SHULL for suggesting this problem, and for constant aid and encouragement throughout the process of its solution.

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OEDOGONIUM NEBRASKENSIS, SP. NOV.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 354

HIRO OHASHI

(WITH TWENTY FIGURES)

In April 1919, Dr. ELDA WALKER found an *Oedogonium* which seemed to be new. The late Professor COLLINS of Tufts College made a preliminary examination of the material and thought the species might be new, but he died before he had an opportunity to make a thorough study. I wish to express my thanks to Dr. WALKER, who kindly turned the material over to me.

I am indebted to Professor CHARLES J. CHAMBERLAIN for criticism and suggestions; and also to Professor E. N. TRANSEAU and Dr. H. TIFFANY, who examined the material and corrected my taxonomic description of the new species.

Material and methods

The following description of the location where the material was collected was supplied by Dr. WALKER:

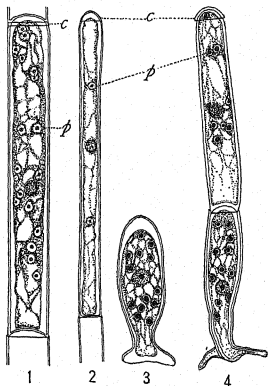
The material was collected April 22, 1919, in the small ponds along a branch railroad line running into the State Fair grounds at Lincoln, Nebraska. The pond from which most of it came was about 50 ft. long and 10 ft. wide. The deepest parts measured hardly a foot. It was a few rods east of the northeast corner of the Fair grounds enclosure. The ponds were full of grasses and prairie plants as they are dry most of the year and seldom retain water any length of time. That was a wet spring and the water stood in the ponds for some time.

The material was killed, fixed, and preserved in a chrom-alum solution: water 100 cc.; chrom alum-2 gm.; formalin 1 cc. It was washed with running water and stained with iron-alum haematoxylin, and also with Magdala red and anilin blue; then it was mounted in Venetian turpentine.

Morphology

The aquatic filament is very small, about 1.5 inches in length, adhering to the surface of grass blades or stems of other plants, giving them a loose velvet-like appearance. It is dioecious, anthe-

ridia and oogonia occurring on separate filaments. The female plant consists of a single series of uninucleate cells, numbering 20-40 (occasionally about 50), including vegetative cells, androsporangia, oogonia, and suffultory cells. The prevailing number is 21-25. The male filament or dwarf male is very much smaller than the female, consisting of just a few cells; so the species is nanandrous.



FIGS. 1-4.—Fig. 1, vegetative cell with reticulate chromatophore, nucleus, and pyrenoids; fig. 2, terminal cell with cap; figs. 3, 4, sporelings showing basal cell with holdfast; $\times 780$.*

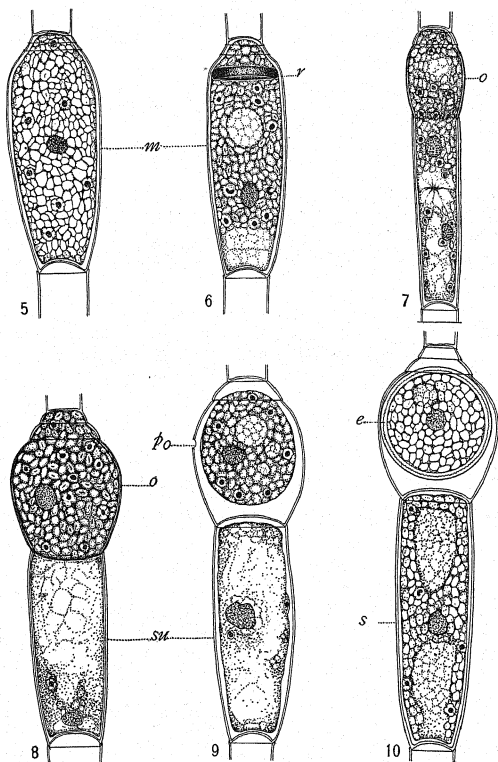
*The following abbreviations are used in all the figures: *a*, androsporangia; *an*, antheridium; *c*, cap; *e*, oospore; *m*, mother cell; *o*, oogonium; *p*, pyrenoid; *po*, pore; *r*, ring; *s*, sperm; *sm*, suffultory cell.

VEGETATIVE CELLS

The vegetative cells are cylindrical, containing peripheral reticulate chromatophores, along which many pyrenoids are scattered (fig. 1). Each pyrenoid is usually surrounded by starch grains. The vegetative cells are slightly capitellate, the terminal cell being attenuate and obtuse (fig. 2), while the basal cell is elongated and has a well developed holdfast (fig. 4). The zoospore germinates into a basal cell (fig. 3), which gives rise to a whole filament by the special type of cell division characteristic of *Oedogonium*, forming at the upper end of the cell a circular ring which makes a new cell wall for the daughter cell. As the result of this kind of cell division, many caps are formed at the upper extremity of the cell.

REPRODUCTIVE CELLS

OOGONIUM.—The formation of the oogonium is started by swellings of the usual vegetative cell which is called the mother cell (fig. 5), and is followed by the appearance of a circular ring at the top (fig. 6), and then by a division of the mother cell (fig. 7). By



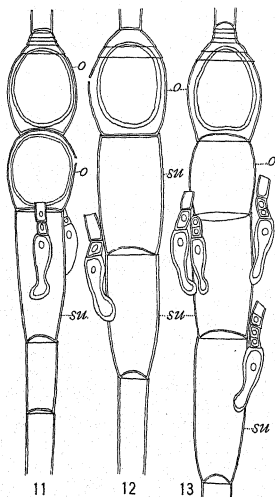
FIGS. 5-10.—FIG. 5, mother cell of oogonium and suffultory cell; fig. 6, ring in mother cell; fig. 7, cell division of mother cell; fig. 8, young oogonium; fig. 9, formation of pore and oosphere; fig. 10, oospore with three layers of cell wall; $\times 780$.

this division the upper one of the two daughter cells grows into an oogonium, while the lower one is a suffultory cell. As soon as the wall of the oogonium is formed by the rupture of the circular ring, the greater part of the contents of the mother cell moves into the

oogonium, leaving large vacuoles behind. Then a cross wall is formed and the oogonium begins to swell (fig. 8.)

After attaining a certain size, a pore is formed at the supramedian part of the oogonium wall, and the contents of the oogonium begin to constrict and round off, forming a single oosphere (fig. 9). Then a sperm from the dwarf male enters the oosphere through the pore and fertilization takes place. After fertilization the wall of the oospore is formed, with three layers, the outer and inner layers which are smooth, and a median layer which is punctate (fig. 10).

The number of oogonia is usually one in a filament, and it rests upon a single suffultory cell (fig. 10), but it is not rare to find two oogonia on a single suffultory cell (fig. 11), or on

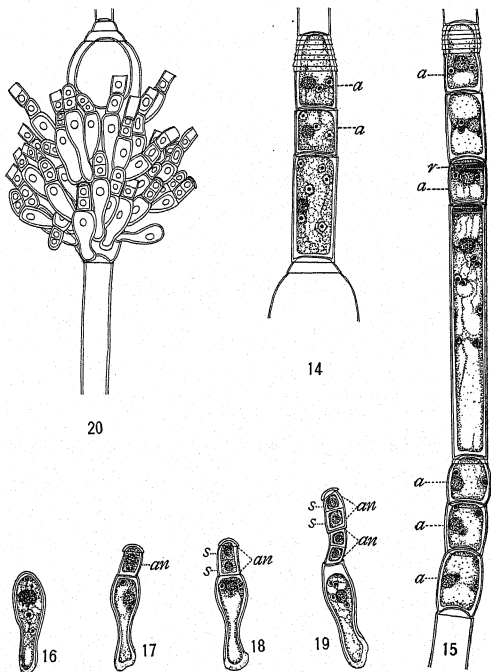


FIGS. 11-13.—Fig. 11, two oogonia on one suffultory cell; fig. 12, one oogonium on two suffultory cells; fig. 13, two oogonia on two suffultory cells; $\times 480$.

double suffultory cells (fig. 13), and sometimes there is a single oogonium on two suffultory cells (fig. 12). Occasionally several oogonia arise on the same filament, separated from one another or occurring in immediate succession. The oogonia are flask-shaped or oviform; the oospores, not quite filling the oogonia, are mostly subspherical or oviform (figs. 11-13), rarely quite spherical (fig. 10).

ANTHERIDIUM.—The dwarf male is produced by germination of the androspore which came from the androsporangium. The andro-

sporangia, occurring in the female filament at a short distance above the oogonium, are much smaller than the vegetative cells (fig. 14).



FIGS. 14-20.—Figs. 14, 15, androgynous sporangia; figs. 16-19, development of dwarf male; $\times 780$; fig. 20, suffultory cell surrounded by more than thirty dwarf males; $\times 480$.

They are either single or double, sometimes three or four in a series (fig. 15). When androgynous sporangia are developed on the female plant

the filament is called "gynandrosporous." The motile ciliated androspores escape from the androsporangia, and after swimming around for a while, attach themselves to the suffultory cell and begin to develop into dwarf males.

The androspore forms first a basal vegetative cell, the so-called stipe (fig. 16). The stipe then gives rise to one or two, rarely three antheridia by typical *Oedogonium* cell division; this type of formation of the antheridia is named "antheridium exterior," one being placed above the other (figs. 17-19). It is very common to find

TABLE I

NAME OF CELL		O. CONCATENATUM f. HUTCHINSIOE	O. NEBRASKENSIS (μ)	REMARKS
Vegetative cell	Diameter.....	26-35 μ	20-35	Very close
	Length.....	3-6 diam. long.	57-236	Some difference
Suffultory cell	Diameter.....	37-50 μ	41-58	Close
	Length.....	1 $\frac{3}{4}$ -4 diam. long.	93-130	Some difference
Oogonium	Diameter.....	52-75 μ	60-67	Rather close
	Length.....	67-95 μ	70-79	Slight difference
Oospore	Diameter.....	50-73 μ	53-64	Close
	Length.....	55-77 μ	60-76	Close

several dwarf males on a suffultory cell; however, in one case thirty dwarf males were seen surrounding a single suffultory cell (fig. 20).

Taxonomy

Measurements of all kinds of cells in *O. nebraskensis* are given in the technical description of the species. The new species is very close to *O. concatenatum* f. *Hutchinsioe* (Wittr.) Hirn, from which it differs in dimensions, in having a punctate spore wall rather "scrobiculate," and in having the pore supramedian instead of "superior." Table I compares the dimensions of some cells of both species.

As shown in table I, *O. nebraskensis* is different from *O. concatenatum* f. *Hutchinsioe* in the dimensions of the cells. The oogonium and the oospore of both species are not so dissimilar in their diameters and lengths. The diameters of the vegetative cell and of the

suffultory cell are also not so different, but the length of both cells in *O. nebraskensis* is somewhat different from that in the other species. Another characteristic of this species is the marking of the cell wall of the oospore, in which there are three spore coats, with the median coat punctate; while that of *O. concatenatum* f. *Hutchinsiae* is "scrobiculate." One more outstanding character of this *Oedogonium* is the position of the pore in the oogonium. This is supra-median instead of "superior," which is represented in the other species with which it is compared. The following is a technical description of the new species.

Oedogonium nebraskensis Ohashi, sp. nov.—Dioecious, nanandrous, gynandrosporous; oogonia single or 2-4, flask-shaped or oboviform, pore supramedian; oospore subspherical or oviform, not quite filling the oogonium; outer spore wall smooth, median wall punctate, inner wall smooth; suffultory cell swollen; androsporangia 1-4-celled; basal cell elongate; terminal cell attenuate obtuse; dwarf males curved on suffultory cell; antheridium exterior, 1-3-celled; vegetative cells slightly capitellate; whole filament 20-41 celled (occasionally about 50).

VEGETATIVE CELLS

	DIAMETER IN μ	LENGTH IN μ
Terminal cell.....	9-12	170-305
Median cell.....	20-27	57-160
Basal cell.....	22-35	79-230

REPRODUCTIVE CELLS

Suffultory cell.....	41-58	93-130
Oogonium.....	60-67	70-79
Oosphere.....	53-64	60-76
Androsporangium.....	20-25	22-40
Stipe of dwarf male.....	12-19	47-68
Antheridial cell.....	9-13	17-20

Summary

1. It might be better to treat this material as a new variety of *O. concatenatum*, so far as the dimensions of the oogonium and oospore and the diameter of the vegetative and suffultory cells are concerned, because there is not enough difference in dimensions to establish a new species. We cannot overlook, however, the differ-

ence existing in the length of the vegetative cells and of the suffultory cells in both species.

2. The marking of the median spore coat of the oospore of *O. nebraskensis* is different from that of *O. concatenatum* f. *Hutchinsioe* in being "punctate," while the latter is "scrobiculate."

3. The most distinguishing character of this species is the position of the pore in the oogonium, in the supramedian position instead of the superior.

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GERMINATION OF SPORES AND EARLY STAGES
IN DEVELOPMENT OF GAMETOPHYTE OF
MARCHANTIA POLYMORPHA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 355

SISTER MARY ELLEN O'HANLON

(WITH PLATES XII-XV)

This study was undertaken to determine whether there is a definite apical cell which acts as the initial in the development of the gametophyte of *Marchantia polymorpha*, as has previously been stated. LEITGEB¹ states: "In reference to the already variously studied germination, one might only mention that with all the variety in the development of the first stages, there is always finally formed at the apex a two-faced apical cell." CAMPBELL² says: "In some of the thallose forms, e.g., Marchantiaceae, Anthocerotes, a single initial cell is not always to be recognized in the older thallus, but in these forms a single initial always appears to be present in the early stages."

Material

The spores for this investigation were collected by Miss CAROLINE WEBER, at Pelican Lake, Oneida County, Wisconsin, during the summers of 1923 and 1924. At that latitude (45° 30' N), the season for spore dispersal begins during the latter part of July, and spores may be collected even later than September 15. At Sinsinawa Mound, Grant County, Wisconsin, about 3° farther south, the season for ripe spores begins in early July and collections may be made toward the end of August. Plants kept under greenhouse conditions shed their spores as early as May 15.

For study of the differentiation of elaters and sporogenous tissue, *Marchantia* plants were collected at Sinsinawa Mound. In the development of the intracapsular tissue of the sporophyte, all of the cells are fundamentally sporogeneous. Later certain cells divert and

¹ LEITGEB, H., Untersuchungen über die Lebermoose 6:121. 1881.

² CAMPBELL, D. H., Mosses and ferns. 2d ed. 1905 (p. 15).

are destined to metamorphose into elaters. The elaters are discernible in an incipient stage five generations before the spore mother cells appear; that is, the diverted cells are sisters to those sporogenous cells which further undergo five series of divisions before they reach the spore mother cell stage. It is estimated that in *M. polymorpha* there are 32 spore mother cells to each elater, therefore 128 spores to each elater.

Spores

The spores of *Marchantia* are exceedingly numerous, although very small. In order to calculate the spore output of a single head, the writer examined over 100 archegonial heads. The number of groups of archegones ranges from 6 to 10, the average and dominant number being 8. Fig. 1 shows that the number of archegones in each group is up to 16, cases having been observed in which there were 18 archegones in a single group.

Although most of the fertilized eggs sometimes undergo a series of cell divisions and sporophyte embryos begin development (fig. 2), it is not likely that more than three sporophytes of a single group, and those nearest the food supply, ever come to maturity (fig. 3). The number of spores in a single capsule is reckoned as not less than 300,000. With an average of 24 mature sporophytes to a head, the total spore output for a single head of *M. polymorpha* is more than 7,000,000 spores.

Methods

Of the various substrates upon which germination was tried, such as soils, wood ashes, charcoal, liquid media, and porcelain plates kept moist with Knop's solution in glass chambers, the last was much the best. This method is superior not only for the growing facilities which it affords, but also for subsequent preparation of the sporelings, which in *Marchantia* are exceedingly small, due to the minuteness of the spores. Spore cultures tried out in the greenhouse were relatively unsuccessful because of the intrusions of algae. Satisfactory results were obtained with cultures grown indoors in east windows of a white walled room with north, south, and east exposures.

Spore germination

The spores of *Marchantia polymorpha* germinate readily upon being shed or shaken from the heads, and they are 100 per cent viable for about a year when kept in envelopes at room temperature. After a year's elapse, the spores rapidly lose their vitality, so much so that spores 14 months old were probably not more than 50 per cent viable; those 17 months old failed totally to germinate. Germination is almost immediate after the spores are sown, and ordinarily begins by a uniform enlargement accompanied by chlorophyll development, until the diameter of the germinating spore is about double that of a freshly shed spore (fig. 4 *a-b*). In the method of germination, and more especially in the subsequent development of the sporelings, however, there is considerable diversity of activities, as well as marked unevenness in the degree of advancement within the same length of time. These differences are influenced particularly by environmental conditions, but also by a certain capriciousness which seems to be a subjective quality of the sporelings themselves, as there is more or less variety in the forms which appear in a single culture. In most cases, a rhizoid appears after a uniform growth of the spore and simultaneous development of chloroplasts have occurred (fig. 4 *c*). Occasionally a second rhizoid appears early, even in the 2-celled stage, the spore giving rise to both (fig. 4 *g*). The second rhizoid from the spore cell may not appear, however, until the young gametophyte has advanced considerably (fig. 5 *g*).

Early stages in gametophyte development

The first cell divisions are usually in one plane only, until a row of cells is formed. The number of cells in the filament is increased under weak light conditions, and accordingly divisions in a second plane are retarded. In light of considerable intensity and long periodicity, divisions in the second plane occur most frequently when the filament is but three or four cells in length. Figs. 4 and 5 show the variety of forms which appear in these early stages. Thus cell divisions, alternating with periods of growth, continue rapidly. Then there comes a time, while the plant is still young and limited to probably less than a dozen cells, when a marginal row of cells appears in the apical region of each plant or branch of each plant, ac-

cording as branching occurs earlier, as it frequently does (fig. 6 b-f). This marginal row of embryonic cells is conspicuous throughout subsequent development, once it is established. The writer examined hundreds of young plants in all stages of germination and growth under a variety of environmental conditions. There were relatively few that showed anything like a wedge-shaped, or otherwise characteristically shaped cell to which any significance as to its potentiality might be attached. Occasionally, in very early stages, before the marginal row is established, there appears an end cell which challenges attention (fig. 6 a). There are many more sporelings, however, that manifest a marked individuality in their course of development, rather than anything like a regular procedure. LEITGEB shows only one figure to support his theory. This is not sufficiently convincing, and one is tempted to conclude that too few specimens were examined. Moreover, the technique available for his extensive investigations on the liverworts was primitive as compared with our present facilities.

In the activity of the embryonic row of cells at the margin of the young plant, differential growth brings about a characteristic notch in the apex (figs. 8-10). In this marginal row of cells, the sum of their outer walls must necessarily exceed in length the combined widths of their inner walls. It is plain, therefore, that the cells of the periphery, some of them at least, must incline to a wedge shape. It is impossible, however, to reconcile the position, form, and apparent function of any cell at the apex of a young thallus so as to designate it as *the* initial cell.

Dorsiventrality is established by the budding off of secondary rhizoids from the side in contact with the substrate. These rhizoids arise somewhat behind the apical notch, and in a line which later becomes the midrib of the young thallus (figs. 11, 12). Like all of the rhizoids which appear in the young gametophytes, these are of the plain walled type. The pegged rhizoids fail to appear until a later stage is reached. Coincident with this, the rise of mucilage cells at the apex insures the anchorage of the young thallus. At about this point too, certain cells, either in the peripheral row or in the outer areas of the plant, manifest themselves as storage organs of essential oils. These cells not only have a characteristic form, and a

diminished size as compared with the other cells of the thallus, but react with osmic acid to indicate their contents (figs. 12, 13).

From the foregoing premises, the conclusion seems to be well warranted that not only is there no single cell upon which the destiny of the plant depends, but that any cell which has retained its vitality is potent to initiate an individual course of action. This individual cell behavior is not alone to send out a rhizoid or to cause a differentiation of parts, but also actually to give rise to a branch which later becomes a distinct plant (figs. 6 e, 7 c, 9 a, 10 a, and 11). Just what part environment or various stimuli may have to play here in determining the course an active cell may take cannot be defined, but fundamentally the response must be due to inherent factors in the cells themselves.

Effects of light, temperature, and moisture

Experiments were tried with spore cultures in daylight prolonged with electric light or shortened by darkening, so that many gradations of light periodicity were devised, under conditions which, other than light, were identical. In the beginning, it was observed that the plants in light periods longer than 16 hours and up to 24 hours advanced more rapidly than those subjected to shorter light periods. This condition prevailed for about three weeks from the date the spores were sown, after which there was a decline in the growth of the sporelings subjected to the long or continuous light periods. At the same time there was a steady increase in the growth of the sporelings which were exposed to 9-16 hours of illumination. The optimum light periodicity for steady and persistent growth seems to be 13-15 hours. Spore cultures placed in weak light, with north exposure, germinated but seemed to produce algal-like forms (fig. 14).

On September 8 experiments were made with cultures, some set up indoors and others out-of-doors. The plants grown out-of-doors were probably a little slower at first, but after the germination stage they much surpassed those of the indoor cultures. This indicates that light of moderate intensity is better for germination, while light of higher intensity is more satisfactory later on. Again, sparse sowings are more successful than profuse sowings, probably more because of shading in the case of the latter than for any other reason.

Figs. 15 and 16 show samples of the indoor and out-of-door cultures respectively as they appeared on September 24, just 16 days after the spores were sown. Figs. 17 and 18 show samples of the same cultures on October 1. It is plain that the out-of-doors and therefore better light conditions are more favorable to the growth of the plant, and this in spite of the fact that higher temperature (which prevails indoors) is better for vegetative growth. This latter point was demonstrated by cultures which were transplanted to soils in flower pots and kept under greenhouse conditions all winter. Here the light intensity and periodicity were the same for plants, some at 10°-15° C. and others at 18°-22° C. Those kept at the higher temperature much exceeded the others in vegetative growth, but fruited much less abundantly than those in the cooler section of the greenhouse. Temperature, however, is less a factor in both the vegetative and fruiting functions than light, longer periodicity being essential³ for the latter, and decidedly better for the former.

Spores sown in Knop's solution in Petri dishes begin germination as readily as those upon a solid substrate, but the course of procedure is different. The bizarre structures which obtain under such conditions are typified in fig. 19. When a liquid medium culture is kept in weak light, with north exposure, the unsuccessful attempts at thallus development are as shown in fig. 20. It is evident that *Marchantia*, although less capable of withstanding drought⁴ than most of its relatives, is by no means inclined to a hydrophytic habit. On the other hand, the sporelings of *Conocephalum*, when sown on liquid media, continue development through a series of stages which are not unlike those which obtain when its sporelings are sown on a solid substrate. This is true, although the adult plants of *Conocephalum* are much more resistant to drought and appear to be much more strictly mesophytic. As a matter of fact, *Marchantia* is limited to a mesophytic habit throughout, and has a rather narrow range between what borders on drought on the one side and excessive moisture on the other.

³ Certain observations recently made indicate that this is true only in the earlier stages in the development of the antheridia.

⁴ The writer wishes to except the gemmae of *Marchantia*, concerning the rôle of which a later paper will appear.

Summary

1. Spores of *Marchantia polymorpha* are available in this general latitude, depending upon the climatic conditions, from early July to the middle of September.
2. Elaters of *Marchantia* are sister cells of sporogenous cells which undergo five divisions before the spore mother cells appear.
3. The ratio of the number of elaters and spores is 1:128.
4. The total number of spores in a single head is estimated to be about 7,000,000.
5. The spores are viable for about a year.
6. One (rarely two) primary rhizoid appears after the growth of the spore and chlorophyll development has occurred.
7. Under ordinarily good conditions for germination, cell divisions giving rise to a very short filament are followed by cell divisions in a second plane.
8. Branching of the young thalli is not uncommon and sometimes occurs at a very early stage.
9. A marginal row of meristematic cells is early established, and these, rather than a single apical cell, are active throughout subsequent development.
10. By differential growth of the cells in the marginal row, a notch appears in the apical region when the young gametophyte comprises 30-40 cells.
11. Dorsiventrality and anchorage are established by the budding off of rhizoids behind and in a line perpendicular to the apical notch, and by the rise of mucilage cells on the lower side of the apex.
12. Excepting during germination, when more moderate light seems better, 13-15 hours of light of good intensity is the optimum.
13. The optimum temperature conditions for vegetative growth are 18°-22° C., and the optimum temperature conditions for fruiting are 10°-15° C.
14. As a medium for successful germination and growth, a solid substrate is much better than a liquid one.

The writer wishes to express sincere gratitude to Dr. W. J. G. LAND, who suggested this investigation, and under whose direction

it was made, and to Mr. ROBERT G. GUTHRIE, who did all of the photographic work.

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EXPLANATION OF PLATES XII-XV

FIG. 1.—Transverse section of young head, showing numerous archegones in each group; $\times 47$.

FIG. 2.—Segment of transverse section of later stage of archegonial head, showing numerous young sporophytes in each group; $\times 30$.

FIG. 3.—Group of three sporophytes; $\times 107$.

FIG. 4.—Spore germination: *a*, freshly shed spore; *b-l*, various stages in germination and development of young gametophytes; $\times 150$.

FIG. 5.—Typical young gametophytes.

FIG. 6.—More advanced stages of young gametophytes: *e*, young plant showing early branching; $\times 150$.

FIG. 7.—Young gametophytes all showing marginal row of cells at apex; *c*, branching thallus; $\times 150$.

FIG. 8.—Two slightly different forms at about the same stage of development: *a*, type of development where light is less intense; *b*, showing slight dip to form the apical notch; $\times 150$.

FIG. 9.—Later stages in development of thallus: *a*, young gametophyte showing three apices; *b*, young thallus showing apical notch; $\times 150$.

FIG. 10.—More advanced stages: *a*, branching thallus, $\times 150$; *b*, typical unbranched thallus; $\times 100$.

FIG. 11.—Photomicrograph of branching thallus; $\times 125$.

FIG. 12.—Photomicrograph of thallus showing thickened apical notch, two budding cells behind apical notch, and storage cells of essential oils in periphery; $\times 175$.

FIG. 13.—Detail of peripheral region of fig. 12, showing two storage cells; $\times 480$.

FIG. 14.—Gametophytes grown in weak light.

FIG. 15.—Photomicrograph of young gametophytes grown indoors, September 8-24; $\times 40$.

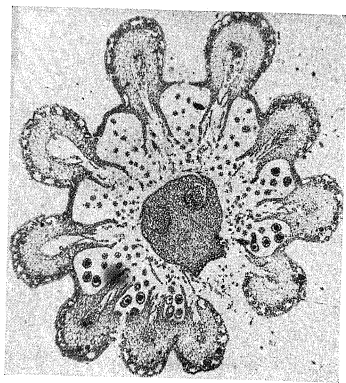
FIG. 16.—Young gametophytes grown out-of-doors, September 8-24; $\times 40$.

FIG. 17.—Young gametophytes grown indoors, September 8-October 1; $\times 40$.

FIG. 18.—Young gametophytes grown out-of-doors, September 8-October 1; $\times 40$.

FIG. 19.—Sporelings grown on liquid medium: *a*, freshly shed spore; *b-f*, various stages of abnormal development; $\times 150$.

FIG. 20.—Sporelings grown in weak light on liquid medium; $\times 100$.



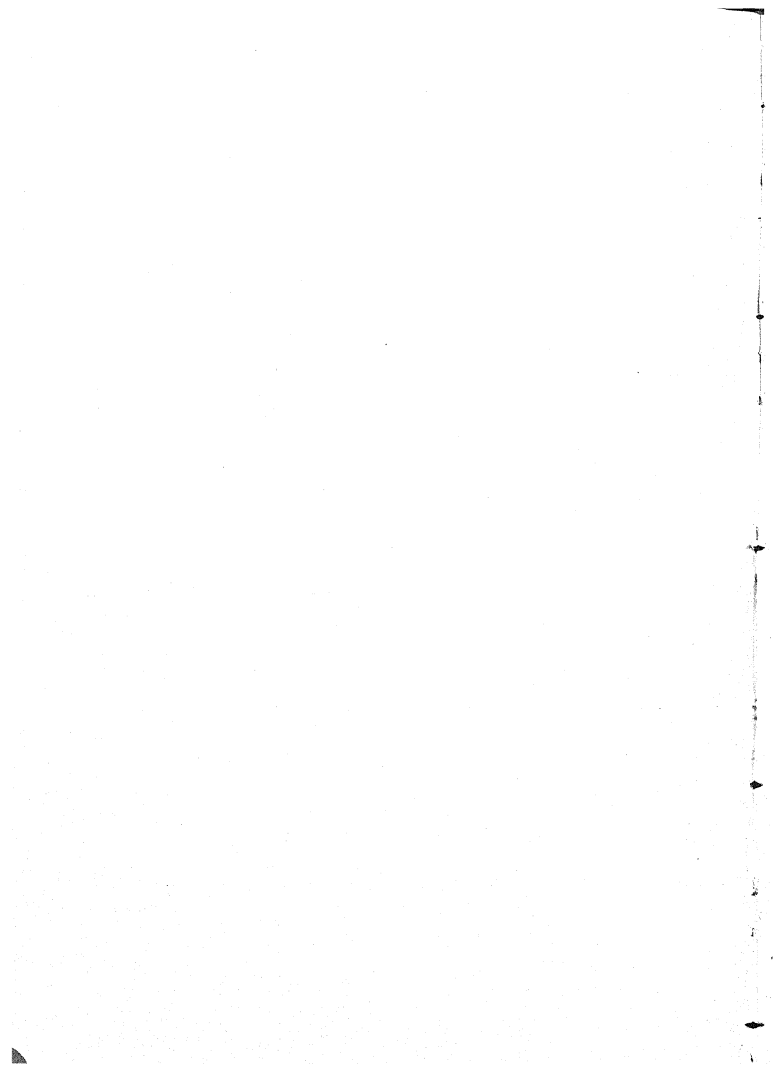
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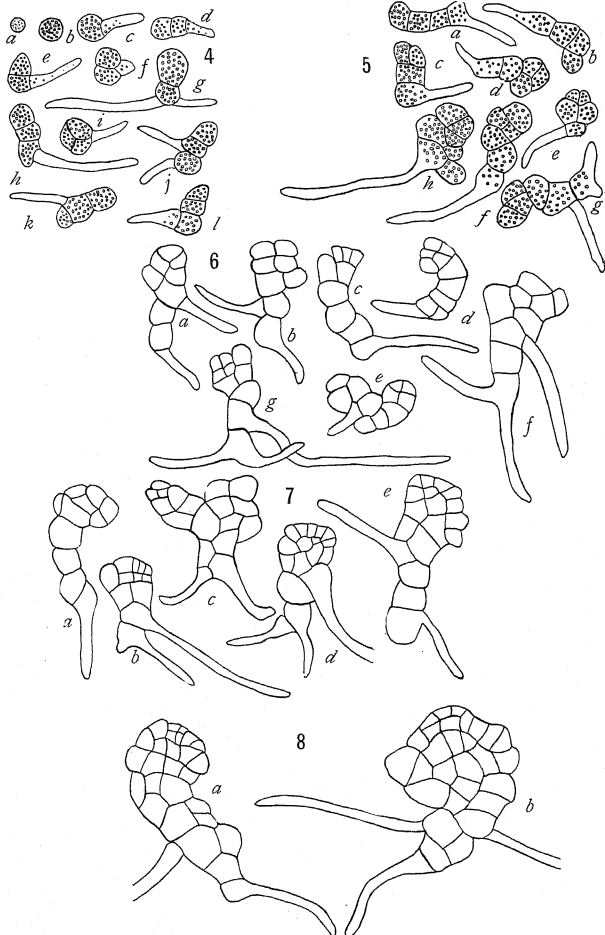


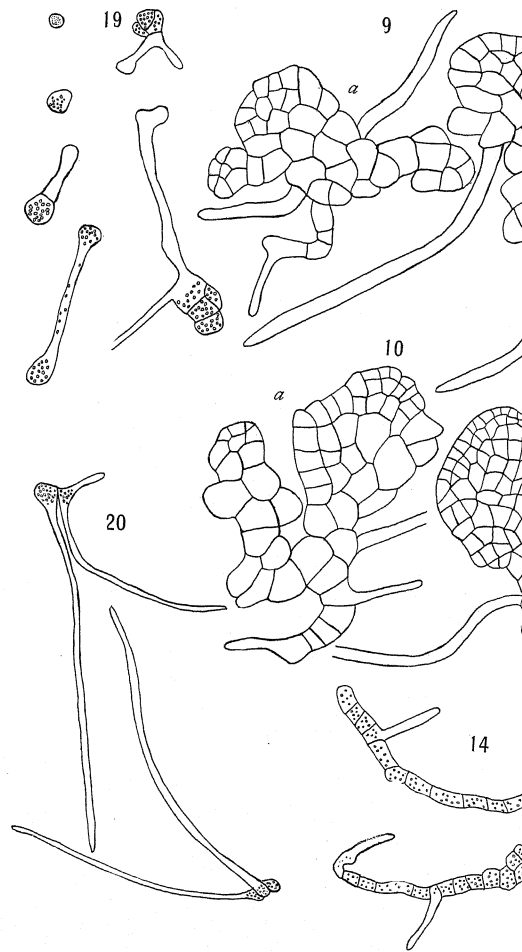
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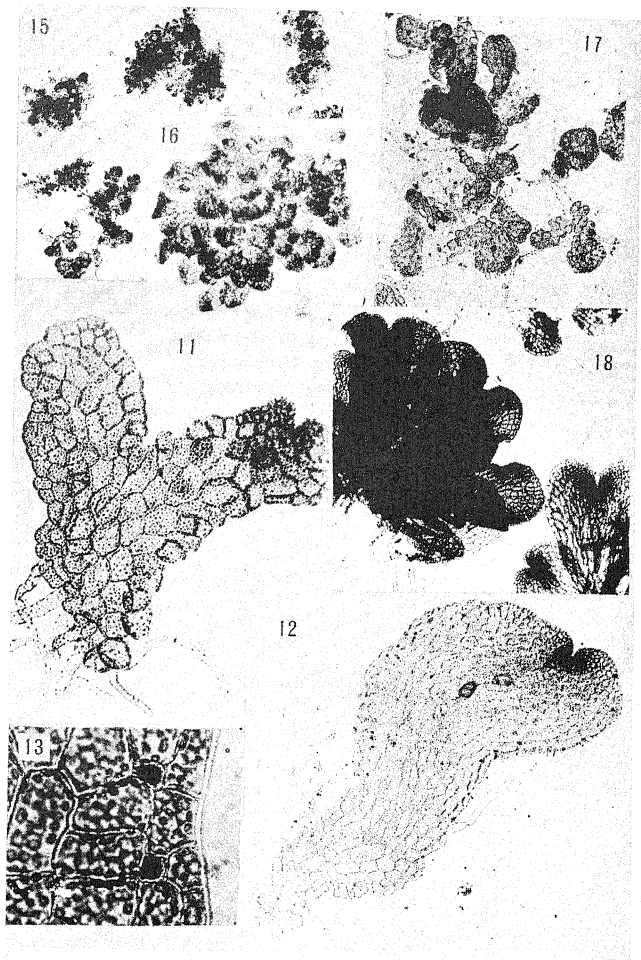


3









BRIEFER ARTICLES

A GENETICAL INTERPRETATION OF ECOLOGICAL ADAPTATION¹

It is well known that different varieties of a given crop may vary greatly in their response to environmental conditions. This adaptation of a variety to more or less local variations in soil and climate is the primary reason for differences in varietal distribution in various parts of the country. Certain varieties are very local in their distribution, while others are found covering a wide range. For instance, the club wheats (*Triticum compactum*) are found almost exclusively along the Pacific coast, while Marquis wheat (*T. vulgare*) is well adapted to much of the territory in northern United States, from Washington to Maine.

Differences in response of a variety to environmental conditions are of considerable importance to the plant breeder. A variety may be developed which is well adapted to local conditions, but which is quite inferior under other conditions, even in the same general locality. The plant breeder's problem may well be illustrated by some of the work at the Maine Agricultural Experiment Station. About sixteen years ago the biologists selected a variety of sweet corn which was productive and of high quality when grown in the vicinity of Farmington, Maine. After the variety had been tested several years it was distributed to representative farmers in different parts of the state. The selected variety continued to maintain its superiority in the vicinity of Farmington, but was relatively unproductive in many localities, and in general was not as desirable as a number of other strains already established. A similar condition was found in the cereal breeding work. The Maine 340 oat, a selection from Irish Victor, is more productive and of better quality than any of the other varieties tested over a period of years. In a season which is generally unfavorable for oat production, however, Maine 340 is less productive than many other varieties. Fortunately conditions in Maine are usually favorable for very high yields of oats, and Maine 340 is very popular and widely distributed among the farmers.

Recent work with certain bean hybrids indicates that differences in adaptation to environmental conditions may be due, in part at least, to

¹ Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, no. 170.

genetic factors which are linked with factors effecting qualitative differences. If certain classes of segregates vary significantly in yield under different environmental conditions, it would seem reasonable to assume that adaptation to environment depends upon the same genetic basis as the segregation of simple qualitative factors, such as pattern and color.

A cross was made between Improved Yellow Eye (I.Y.E.) and a small white bean of local origin. The I.Y.E. is relatively unproductive, and in 1924 yielded 14 gm. per plant as compared with 25 gm. per plant for the white parent. The beans on the F_1 plants were completely pigmented and mottled, indicating that factors for complete pigmentation and for mottling were contributed by the white parent. The F_2 gave a ratio for

TABLE I
YIELDS IN GM. PER PLANT OF SEGREGATES OF CROSS I.Y.E. \times WHITE

GENERATION	WHITE		SELF-COLORED		MOTTLED	
	N	Weight	N	Weight	N	Weight
F_2 1923....	478	8.90 \pm 0.16	241	8.03 \pm 0.19	655	10.05 \pm 0.15
F_2 1924....	79	45.9 \pm 0.17	32	39.1 \pm 2.01	591	39.9 \pm 0.6
F_2A 1925....	590	16.02 \pm 0.25	384	21.05 \pm 0.34	486	19.89 \pm 0.29
F_2B 1925....	585	26.79 \pm 0.36	384	25.17 \pm 0.41	465	30.57 \pm 0.45

27 mottled; 9 self-colored; 12 eyed; 16 white; indicating that the factors for mottling, complete extension of the pigments, and pigmentation belong to independent linkage groups. Each of these characters is dependent upon a single factor difference, and each shows a simple Mendelian segregation.

The entire F_3 was planted in such a way that the various classes of segregates were distributed at random throughout the field. The soil was not particularly well adapted for beans and the season was very unfavorable for bean production. The average yield per plant was about 9 gm. As shown in table I, the average yields per plant for the three classes of segregates differed significantly. Previous work² had already shown that seed size was linked with simple qualitative differences, so that the association of yield with color and pattern differences was not unexpected.³ In F_3 the mottled segregates were significantly more productive than the self-colored ones. Since three-eighths of the white segregates

² SAX, K., The association of size differences with seed coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552-560. 1923.

³ ———, The nature of size inheritance. Proc. Nat. Acad. Sci. 10:224-227. 1924.

should be homozygous for mottling and one-fourth should be heterozygous for mottling, this class would be expected to be, and is, intermediate in productivity as compared with the two pigmented classes of segregates. These results show that yield, to some extent at least, is dependent upon genetic factors which are linked or associated with factors for simple qualitative differences, and, therefore, are inherited in the usual Mendelian manner.

Only F_3 mottled segregates were selected for planting in F_4 . Ten seeds of each F_3 plant were used. The F_4 rows were planted on ideal soil for beans, and the climatic conditions in 1924 were very favorable. The average yield of all classes of segregates was 41 gm. per plant as compared with 9 gm. per plant in 1923. Many of the F_3 mottled plants segregated into mottled, self-colored, and white segregates in F_4 . The mottled segregates of course are far in excess of either the white or self-colored, but sufficient of the last two classes are available to make the comparisons of productivity. As shown in table I, the white segregates were significantly more productive than either the mottled or self-colored segregates. Since the F_4 families which were heterozygous for pigmentation and mottling factors were presumably distributed at random over the plots, there should be no differential effect due to soil heterogeneity in respect to the yields of the three classes of segregates. In order to check environmental effects of location, the white segregates were also compared with only the mottled segregates in the same row. A comparison of white and mottled classes in segregating rows gave practically the same results as a comparison of all white segregates with all of the mottled individuals.

The facts that the mottled segregates were most productive in F_3 , under unfavorable conditions, and that the white segregates were most productive in F_4 under favorable conditions, suggest that the factor for high yield which is associated with the recessive pigmentation factor is effective only under certain environmental conditions, while the factor or factors for productivity which are associated with the mottling factor are effective under other environmental conditions.

If productivity is due to the same genetic factors in successive years there should be some correlation between the yield of the F_3 plants and the F_4 progeny. There is, however, no significant correlation between the yields of the F_3 plants and the F_4 segregates ($r=0.13 \pm 0.07$). This low degree of correlation can hardly be attributed to lack of genetic differences in productivity. The great variation in plant yield is indicated by the coefficients of variability, which are about 50 for each class of segregates in each of the three years. Much of this great variability undoubtedly is

due to environmental factors, but the fact that the coefficients of variability of the white and I.Y.E. parents for 1923 were only 21 and 20, respectively, indicates that considerable variation in yield was due to heredity. Although hereditary differences are apparently involved, there is no significant correlation between the yields of F_3 plants and their F_4 progeny, further supporting the suggestion that hereditary factors were involved which affected the differences in productivity of the various classes in successive years.

In 1925, 30 white, 30 self-colored, and 30 mottled segregates were selected from the F_4 . The seeds of each plant were divided into two parts and designated as *A* and *B* in addition to the selection number. Lot *A* was planted in heavy soil not well adapted to beans, and lot *B* was planted in a better type of soil a quarter of a mile away. Approximately the same number of seeds was planted in the two selections of each F_4 plant. These classes of segregates, white, self-colored, and mottled, were not planted at random in the two plots, although an attempt was made to avoid soil differences between classes by making the plots long and narrow and planting each class of segregates in rows the length of the plot. The arrangement of pattern classes in long rows to avoid the effect of soil heterogeneity was of doubtful value in plot *A*, because when white segregates from F_4 mottled plants were compared with the mottled segregates in the same rows there was no significant difference in yield, although the white segregates from F_4 white plants are less productive than the self-colored or mottled segregates. In plot *B*, however, the mottled segregates were found to be significantly more productive than either the self-colored or white segregates. In both plots, *A* and *B*, the yields per plant are intermediate between the yields found in F_3 and F_4 (table I). The differences in productivity of various classes are not very consistent in F_5 , although perhaps under a still different state of environmental conditions the intermediate yields should not necessarily mean a corresponding rank in productivity of the various classes of segregates. In F_5 the two lots *A* and *B* do not differ greatly in yield, and neither lot shows any significant correlation with the parental F_4 plant ($r=0.20 \pm 0.07$ and 0.01 ± 0.07 respectively). There is some correlation between the two lots of F_5 plants, however, although the correlation ($r=0.37 \pm 0.06$) is not as high as one might expect, considering that the corresponding F_5 rows should be practically identical in genetic constitution, and that the yields per plant do not differ greatly. Although there is some evidence of differential adaptation of yield factors in F_5 , the results are not as consistent as those found in F_3 and F_4 . Altogether, however, there is con-

siderable evidence that the effect of yield factors is dependent on environmental conditions.

The genetic interpretation of adaptation to environmental conditions is also supported by ENGLENDOW'S⁴ recent experiment in spacing tests with cereals. Two varieties of wheat were planted at different distances, from 2 inches×2 inches to 18 inches×6 inches. One of the varieties, Red Fife, yielded about 24 bushels per acre under field conditions, while the other variety, Hybrid H, yielded about 40 bushels per acre. When the plants are closely spaced (2"×2") Red Fife is more productive than Hybrid H. When the plants have a little more space (2"×6") the two varieties are about equally productive, and at greater distances Hybrid H is more productive than Red Fife. It is evident that Red Fife is better adapted to crowding, and that Hybrid H is more responsive to increased opportunities for using the available space. Undoubtedly these differences in response to spacing play an important part in field production. It is also probable that genetic factors are involved in varietal differences of this kind.

Conclusion

In a cross of Improved Yellow Eye×Small White beans, there is a simple 3:1 segregation for pigmented versus white and for mottled versus self-colored in F_2 . These differences in seed coat pattern are associated with differences in yield per plant, indicating that factors for plant yield are linked with these simple qualitative factors (it is possible of course that the same factor affects yield and seed coat pattern). In F_3 , under unfavorable conditions, the mottled segregates were more productive than the self-colored or white segregates; while in F_4 , when all classes were relatively productive, the white segregates were the highest yielders. This behavior suggests that the factors for yield are dependent on environmental conditions for their expression, and that ecological adaptation and distribution of economic plant varieties are dependent on genetic factors.—KARL SAX, *Maine Agricultural Experiment Station, Orono, Me.*

[Accepted for publication January 14, 1926]

⁴ ENGLENDOW, F. L., Investigations on yield in the cereals. *Jour. Agric. Science* 15:1125-1146. 1925.

CURRENT LITERATURE

BOOK REVIEWS

Root development

WEAVER^{*} has produced a book on *Root development of field crops* that will prove of great interest and value to workers in all lines of plant science, including equally those interested in pure and applied research, as well as some practical producers. It is also sure to stimulate investigation in a field that has had far too little attention, and will interest horticulturists, agronomists, and market gardeners in a phase of their problems that has been too little considered.

The chapter headings give a good idea of the scope and organization of the book: the environment of the root; the soil; how roots are built to perform their work; root habits in relation to crop production; root habits of native plants and how they indicate crop behavior. Chapters V-XVI deal with the root habits of wheat, rye, oats, barley, corn, sorghum, various meadow and pasture grasses, sugar beets, alfalfa, various clovers, potato, and sunflower. Chapter XVII describes the methods of studying root development. The bibliography consists of 232 citations, and there is also a comprehensive index of 16 pages.

The reviewer feels that the book is a most judicious statement of the factors affecting root development and of the effect of root development upon crop production. Many factors are considered. First is the hereditary factor. The roots of some plants are very fixed in form, being modified relatively little by environmental factors. The roots of other plants are far more plastic, being greatly modified by environmental conditions. This is generally true of crop plants. The effect of the following environmental factors are rather fully considered: soil and subsoil; water and nutrients in the soil and subsoil, including irrigation of different amounts and times of application, and the application of fertilizers of various sorts to the soil and subsoil; transplanting, tillage, intercropping; rotations of various sorts; and acids, alkalies, temperatures, and oxygen supply. The literature on the effect of conditions upon the susceptibility and resistance of roots to diseases is also brought together.

While many of the data presented are from the extensive work of the author and his associates, all other sources of information have been drawn upon, including agronomy, horticulture, physiology, and pathology. The book is an excellent critical treatise on the information in this field to date. As one reads it, he wishes that every phase of plant science as bearing on practice was as well summarized and as critically treated.—W. CROCKER.

^{*} WEAVER, J. E., *Root development of field crops*. 8vo. pp. xii+291. New York: Hill Book Co. 1926. \$3.00.

MINOR NOTICES

Imperial Botanical Conference

A considerable number of the papers and discussions at the Imperial Botanical Conference² held at London, July 1924, took up matters of pathological and mycological interest.

It is only natural that at a conference attended by botanists from all parts of the British Empire there should be papers on the diseases which affect important crops in the British Isles, the dominions, and the colonies. The following crops were represented: apple, citrus, clove, coconut and other palms, potato, rubber, sandal, sugar cane, tomato, and forest trees and timber. Several papers showed that the marketing and storage of fresh plant food products are presenting problems in England, as in the United States, and that botanical research is being used to cope with them. Under the heading "The biological problems of the cold storage of apples," a series of papers deals with various aspects of the "ontogenetic metabolic drift" of apples in storage, and the relations of various living and non-living factors thereto. The concept of a metabolic drift should prove to be very useful.

There were several papers on the relation of plant pathology to genetics. A plea was made (Dr. WM. B. BRIERLEY) that the plant pathologists and mycologists acquaint themselves with the work of the modern geneticist, so that they may, as much as possible, use the concepts and criteria of the geneticists when they engage in genetical considerations of their problem. It is undoubtedly true that there has been too much of "thrusting results somewhat blindly into the framework of genetics."

It was pointed out that the pathologist and mycologist should proceed cautiously in employing the terminology of the geneticist, especially the term mutant, and take pains to make sure that the material in question is as pure genetically as is the homozygous material of the geneticists. It may be wholly unwarranted, for example, to assume that a single spore or hyphal tip culture is genetically pure.—GEO. K. K. LINK.

Forestry almanac

This useful compilation of facts³ concerning our forests, edited by CHARLES LATHROP PACK, has been much enlarged since the appearance of the first edition was noted in this journal⁴ two years ago. Developments during these two years have included considerable advances in the United States Forest Service, and this edition brings the history of this branch of the Department of Agriculture down to date. Among other data are included information regarding the organi-

²Imperial Botanical Conference, London. July 7-16. 1924. Report of Proceedings. Ed. by F. T. BROOKS. Cambridge. 1925.

³American Tree Association. Forestry Almanac. Semicentennial edition. pp. xiii+348. Philadelphia: J. B. Lippincott Co. 1926.

⁴BOT. GAZ. 78:356. 1924.

zation and activity of some 70 forestry organizations, and of over 20 college and university courses in forestry. Its list of books on forestry is also extensive. In a word, it seems to contain more information regarding forestry and forest conditions than any other book before the public.—GEO. D. FULLER.

NOTES FOR STUDENTS

Taxonomic notes.—DOWNIE⁵ has published descriptions of the new orchids from Siam determined by the late R. A. ROLFE. The collections were made by A. F. G. KERR, and include 35 new species, representing 17 genera.

MERRILL⁶ has published a list of plants collected on Banguay Island, which lies between Borneo and the Philippines. The collection was made by some forest rangers, and is estimated to represent probably only about one-fourth of the flora. The flora of the island as represented in this collection is remarkably intermediate between that of the Philippines and that of Borneo. More than 400 species are listed, and 21 of them are described as new. The specimens are on deposit in the Herbarium of the University of California.

ASHE⁷ has published some notes on the development of pubescence in *Tilia*, and also describes a new species (*T. lata*) from Alabama. He also publishes a key to the species of *Tilia* in eastern United States with lower leaf surface tomentose at time of flowering.

OSTERHOUT⁸ has described three new species from Colorado under the following genera: *Atriplex*, *Sophia*, and *Militzia*.

MERRILL⁹ has published a second paper describing new species from Indo-China, based on material collected by A. PETELOT. It includes 18 new species, and 18 others recorded from Indo-China for the first time. The new species are distributed among 14 families.

In continuation of his investigation of Fabaceae, RYDBERG¹⁰ has presented 2 genera segregated from *Astragalus*, namely, *Geoprunnon* Rydberg, with 7 species, and *Hesperastragalus* Heller, with 12 species, 2 of which are described as new.

NELSON¹¹ has presented "the loco plants," in view of their return to *Oxytropis*. He describes 9 species, one being new, presents 8 other species, and closes

⁵ DOWNIE, D. G., Contributions to the flora of Siam. Bull. Kew Gardens no. 9. 1925 (pp. 367-394).

⁶ MERRILL, E. D., The flora of Banguay Island. Philippine Jour. Sci. 29:341-427. 1926.

⁷ ASHE, W. W., Notes on *Tilia*. Bull. Torr. Bot. Club 53:27-33. 1926.

⁸ OSTERHOUT, G. E., New plants from Colorado. Bull. Torr. Bot. Club 53:35-36. 1926.

⁹ MERRILL, E. D., New species of plants from Indo-China. II. Univ. Calif. Publ. Bot. 13:127-143. 1926.

¹⁰ RYDBERG, AXEL, Notes on Fabaceae. VII. Bull. Torr. Bot. Club 53:161-169. 1926.

¹¹ NELSON, A., Taxonomic studies. Univ. Wyoming Publ. Bot. 1:109-143. 1926.

the account by considering "troublesome pairs and doubtful species," 5 in number, 2 of which are described as new. He also describes and discusses 22 new species in 13 miscellaneous genera, 5 of which belong to *Phlox*, 3 to *Gaillardia*, and 3 to *Senecio*.

PAYSON¹² has presented 3 genera, with full descriptions, citations, and illustrations. The genus *Thlaspi* in North America is recognized as including 6 species, with one new species and 2 new varieties. *Oreocarya* includes 7 species, 5 of which are new. The third genus is considered under the title "*Erigeron compositus* and its allies in the United States," involving the presentation of 4 species (one of which is new) and 4 varieties. He has also published a long list of names that have been used for *Erigeron compositus* and its allies.

RUSBY,¹³ following BITTER's reinstatement and extension of *Lycianthes* (Solanaceae), has published a number of transfers from other genera and also some new species. In BITTER's presentation, over 300 species were transferred from other genera, 250 of them from *Solanum*, so that *Lycianthes* is a very large genus. RUSBY adds 6 new species (from Bolivia and Colombia) and 5 new combinations, from material in the herbarium of the New York Botanical Garden.—J. M. C.

Bud formation.—SUMMERS¹⁴ has summarized the literature dealing with the problem of bud formation in plants. Attention is called to the present great need in horticulture of going beyond the common horticultural practices of pruning, grafting, defoliation, and ringing, and applying the methods of the plant physiologist and the plant biochemist in determining the factors that are really modifying the activity of the plant. The review is a survey of the progress made by the plant physiologist in this field, and is organized about the following main subjects: the sap concentration factor; LOEB's inhibition hypothesis, with evidence for and against it; the food reserves of the shoot; the resting period of buds; ringing experiments; and certain miscellaneous experiments bearing on the problem of bud formation.

Of these various lines of attack, SUMMERS rightly states that the studies having to do with the food reserves and the sap concentration promise to throw more light on the problem of bud formation. The work of KRAUS and KRAYBILL, BUTLER, SMITH and CURRY, HARVEY, and others has been summarized in this section. This part of the review seems rather incomplete, although much of the work done since KRAUS and KRAYBILL's paper in an attempt to get at the chemi-

¹² PAYSON, E. B., *Thlaspi*, *Oreocarya*, and *Erigeron*. Univ. Wyoming Publ. Bot. 1:145-186. 1926.

¹³ RUSBY, H. H., Additions to the genus *Lycianthes* Dunal. Bull. Torr. Bot. Club 53:209-213. 1926.

¹⁴ SUMMERS, F., The factors governing bud formation: a chapter of plant physiology. New Phytol. reprint no. 10 (New Phytol. 23: nos. 1, 2, 3. 1924). London: Wheldon and Wesley. 1924.

cal basis of vegetation and reproduction was probably published too late to be included in the review.

While the study of the chemical situation in the plant seems to give the greatest promise of explaining the problems of correlation and bud formation, it must be admitted that much further work remains to be done before we have a satisfactory explanation of these problems. It may also well be that stimuli, hormones, and inhibiting substances will have a place when the full story is told.

MISS FERNALD¹⁵ has published a recent paper on the subject of bud development. She finds that there is a rather close correlation between the osmotic concentration of a tissue and the tendency of this tissue to inhibit the development of other tissues or be inhibited by them. For example, it was found that a potato with a strong terminal sprout nearly always showed a higher concentration of the sap of the apical eyes than of the more basal eyes. Also shoots of privet and *Philadelphus* whose terminal buds were actively growing, and which did not develop lateral branches the first year, generally showed a more concentrated sap in the apical portions than in the more basal portions. The author realizes that while osmotic concentration may be an important factor inhibiting bud development, it is only one of the possible factors involved.—S. V. EATON.

Flora of the Black Hills.—This bulletin¹⁶ is devoted primarily to descriptions of the geologic formations of the Black Hills and to the mineralogy and economic geology of the region. It includes, however, a section on the flora of the State Park by A. C. MCINTOSH, Assistant Professor of Biology at the State School of Mines, who has recently made extensive collections of the plants of the Black Hills, including more than 400 species from the State Park. Some 200 of these are listed under the following habitats: foothills, foothills streams, mountains, moist ravines, valleys, the gorge and granite peaks.—H. E. HAYWARD.

¹⁵ FERNALD, EVELYN I., The inhibition of bud-development as correlated with the osmotic concentration of sap. *Amer. Jour. Bot.* 12:287-305. 1925.

¹⁶ O'HARRA, C. C., and CONNOLLY, J. P., The geology, mineralogy, and scenic features of the Custer State Park, South Dakota. S.D. School of Mines. Bull. no. 14.

THE
BOTANICAL GAZETTE

November 1926

VASCULAR TISSUES OF MICROCYCAS CALOCOMA

MINTIN A. CHRYSLER

(WITH PLATES XVI-XVIII AND THREE FIGURES)

Microcycas is the most accessible of the treelike cycads to a student in this part of the world, yet its structure and life history are imperfectly known. The extremely narrow range of this monotypic genus, and the apparently small number of individuals render a careful study desirable. With the special object of securing properly preserved material of the phloem of an arboreal cycad, a journey to western Cuba was undertaken in the summer of 1924. In this connection it is a pleasure gratefully to acknowledge the favor of a grant from the research fund of the New York Academy of Sciences. Acknowledgment is also made to the following persons, who have supplied material or aided in its collection: Professor C. J. CHAMBERLAIN of the University of Chicago, Director N. L. BRITTON of the New York Botanical Garden, Dr. O. W. CALDWELL of the Lincoln School, New York City, Director G. W. FORTUN of the Estacion Agronomica of Cuba, Dr. J. T. ROIG, Botanist of the same station, Mr. H. A. VAN HERMANN, Santiago de las Vegas, Cuba.

Field observations

The plant was studied at two stations, San Diego de los Baños and Consolacion del Sur, both in the province of Pinar del Rio, western Cuba. In the neighborhood of San Diego the plant was found on the slopes of the siliceous rounded hills locally known as "lomas." The station near Consolacion, however, is in nearly level

country south of the Santa Catalina range, the plants occurring to the number of about a dozen along a small water course. CALDWELL'S (2) observations indicate that "the plants are closely limited

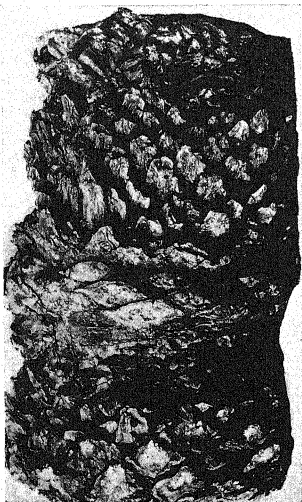


FIG. 1.—Portion of trunk, showing wide zones covered with scales and narrow zones occupied by leaf bases; $\times 0.5$.

in elevation, the level showing them to be at essentially the same elevation, although they vary greatly in their distances from the tops of the mountains upon which they grow." The healthy appearance of the specimens at Consolacion, and the presence of a cone on nearly every individual show that a high altitude is not necessary for the proper development of the plant. At the same time, no seeds or young seedlings were found, so it is improbable that the species is extending its range. Provided suitable conditions are present, the plant shows good capabilities for vegetative reproduction, for instance by sprouts. At San Diego a specimen was observed

which had been bent over and had assumed an S shape, with an extra crown arising at the summit of the convex arm of the S.

So far as can be ascertained, the total number of individuals within the very restricted range is decidedly small, and the plant is sought for several reasons.¹ The extremely slow growth of the plant,

¹ The local name "palma corcha" is applied because of the use of the seeds as bottle corks.

its occurrence in small detached groups, and the existence of solitary aged specimens, as recorded by CALDWELL, plainly indicate that this rare plant is rapidly nearing extinction. It was with much satisfaction, therefore, that the writer learned that the Cuban government has lately taken action in prohibiting the removal of specimens of the plant.

Trunk

The considerable height (up to 30 feet) of mature specimens has been recorded by CALDWELL (2) and CHAMBERLAIN (6). The measurements in the present paper are from a specimen about ten feet high, which was cut down at the station near Consolacion del Sur. Both of the writers just named figure the conspicuous rings or circular ridges which occur rather irregularly at intervals of a few inches. In the upper part of a trunk it is easy to see on the rings the scars left by the fallen leaves, which have been cut off by an absciss periderm, as described by CHAMBERLAIN for *Dioon* (5). The wide spaces between the rings are occupied by the ragged bases of scale leaves. The general appearance is shown in fig. 1, taken from a piece of stem somewhat more than a foot below the apex. A comparison with CHAMBERLAIN's figure of the trunk of *D. spinulosum* (5) shows that in the latter genus the relation between leaf bases and scale bases is reversed, namely, the zones of leaf bases are the broad ones, and the zones of scales are quite narrow. It is obvious that in *Microcycas* the number of ridges indicates the number of crowns which have been produced by the plant. If one could be certain as to the frequency with which the new crowns are produced, it would be easy to approximate the age of a specimen, for the ridges are quite persistent. A reliable observer at the Experiment Station at Santiago de las Vegas reports that the four plants which are growing at the Station produce a crown every two years. Dr. JUAN T. ROIG has very kindly undertaken to measure the intervals between the rings on the trunks of the four specimens just mentioned, and his measurements yield the following figures:

- Plant no. 1, 19 rings in distance of 9 ft. 4 in.; average interval 6 in.
- Plant no. 2, 37 rings in distance of 9 ft.; average interval 3 in.
- Plant no. 3, 18 rings in distance of 6 ft.; average interval 4 in.
- Plant no. 4 (less than 5 ft. high).

These figures give a value of almost exactly 4 inches as the average distance between rings in specimens growing under rather favorable conditions. If a new crown is produced every two years, a plant obviously increases its height by 2 inches per year, or a tree 10 feet high would be somewhat over 60 years old, since the growth in height is less rapid during the first few years of the plant's life. Such an estimate corresponds fairly with CHAMBERLAIN's figure for *Dioon spinulosum*, where a tree 20 feet high is calculated to be over 100 years old, on the assumption that a crown is produced every year.



FIG. 2.—Part of transverse cut through trunk, showing wide pith and cortex and narrow vascular ring with rays; cortex shows a few of the girdling leaf traces; $\times 0.66$.

A section taken transversely through the trunk shows the usual cycadean condition of an exceedingly wide pith, a narrow vascular ring, and a broad cortex surrounded by the bases of leaves or scales (fig. 2). It will be observed that the trunk of *Microcycas* is monoxyletic, in contrast with such genera as *Cycas* and *Macrozamia*. Longitudinal cuts through the trunk display also at intervals the "cone domes" or vascular supply of cones, corresponding to CHAMBERLAIN's fig. 4. It has not been possible in *Microcycas* to match these cone domes with the external rings. The following measurements are contributed for comparison with those furnished by CHAMBERLAIN (5): in a trunk of *Microcycas* about 3 m. high, at a distance of 300 mm. from the apex the diameter of the trunk was 110 mm., pith 65 mm., thickness of xylem 4 mm., of phloem 2 mm., and of cortex

16 mm.; while at a distance of 2500 mm. from the apex the diameter of the trunk was 120 mm., pith 72 mm., thickness of xylem 9 mm., of phloem 5 mm., and of cortex 10 mm. These figures correspond very nearly with those for all but the very old stem of *Dioon*.

Histology of stem

TECHNIQUE.—Material was fixed soon after collection in JEFFREY's corrosive sublimate-picric acid mixture, which was found to be entirely suitable. In the early part of the study the abundant starch contained in the stems was partly removed by digesting at 50° in diastase solution, but it was later found that the material imbedded readily in celloidin without preliminary digestion, after treatment with hydrofluoric acid. With material thoroughly infiltrated, no difficulty was experienced in cutting sections sufficiently thin for photographing. The sections were stained in Haidenhain's haematoxylin, followed by safranin, mounted in balsam, and finally flattened by spring clothes pins.

XYLEM.—In general the xylem of *Microcycas* corresponds in structure to that of the other monoxyle genera, namely, it consists of tracheids, wood parenchyma, and rays, certain of the last carrying leaf traces. The general relations of these as seen in transverse section are shown in fig. 4. The parenchyma cells may clearly be distinguished from the tracheids by the single contour of the former. The essential similarity of this wood to that of *Dioon* may be seen by comparison with CHAMBERLAIN's fig. 20, in which case, however, no parenchyma cells are shown, although they occur plentifully in that genus, as shown by independent observation, and indicated by CHAMBERLAIN's fig. 17. The woody elements are grouped in narrow radial strips from one to about four cells in width, separated by the rays. The tangential diameter of the mature tracheids is about 45 μ , and the length varies widely, ranging from 3.7 to 8 mm., with average at 6 mm. These measurements correspond closely to those taken by Miss LANGDON in her study of the stem of *Dioon* (9), and show that the tracheids of cycads are definitely longer than are those of conifers, for the average length of the tracheids of 29 species of conifers listed by RECORD (14) is 3.77 mm.

The nature of the protoxylem in cycads has been called in ques-

tion, and even its occurrence in the adult stem has been doubted. One reason for difficulty in finding protoxylem in an old stem, as METTENIUS (11) pointed out and figured long ago, is the eventual fragmentation of the inner layers of xylem owing to the growth and division of parenchymatous cells in the outer region of the pith (fig. 15), leading not only to separation of the elements, but also to throwing many of them out of perpendicular. MILLER (12) correctly states that the early tracheids pursue an undulating course, which makes them hard to follow (this however does not apply to a tangential section). In material taken from within a foot of the stem apex in *Microcycas*, however, it is easy to be certain that one is dealing with actual protoxylem. The tracheids at the tip of one of the xylem wedges shown in figs. 14 and 23 fulfil at least two of the requisites for being regarded as protoxylem. (1) They are narrow, their diameter being about 10 μ , while that of the tracheids of the mature secondary wood ranges around 45 μ . (2) They are not heavily lignified, as shown by the phloroglucin test, but they rarely show a steep or broken spiral thickening, and are rather characterized by a close spiral thickening which quickly merges in later-formed tracheids into a reticulate or scalariform thickening located on all faces of a tracheid, but devoid of border. The absence of conventional protoxylem with steep spirals might be accounted for by the supposed collapse or destruction of such elements during growth of the stem. METTENIUS endorses such an explanation in the case of *Cycas revoluta*, and describes ring and spiral tracheids in the inner part of the stele of a sufficiently young plant. MILLER, however, finds in *C. media* that "protoxylem elements are usually scalariform, although hints of spiral tracheids are more or less frequent." He observes, moreover, "since neither transverse nor radial preparations show crushed masses of cellular material at the centripetal ends of the bundles, there can be no doubt that the xylem elements which can be seen to terminate the bundles are truly protoxylem, whether they are spiral or scalariform." In other words, it is the nature of a cycad to produce protoxylem of the scalariform rather than spiral type in its stem. It will be seen later that the petiolar bundles of *Microcycas* and other genera show a protoxylem with definite spirals which are more or less disorganized, as though pulled out. It might

be argued that the cycad leaf undergoes great extension, while the stem lengthens very slowly, but the absence of steep spirals in the rapidly elongating leader of pine or fir casts much doubt on this explanation. The stresses present in the stem tip of cycads appear to be due to pressure rather than tension, owing to the great growth of the pith, leading to the pushing asunder of the primary tracheids, as was pointed out by METTENIUS, and as may be discovered in material of *Microcycas* cut several feet below the stem tip (fig. 15). On the whole it appears safe to conclude that the protoxylem of the cycad stem is of a somewhat advanced or abbreviated type, in which the spiral elements are few and the scalariform condition is quickly reached.

Contrasting with the precocity just credited to the protoxylem is the extreme slowness with which the secondary tracheids attain their typical or adult form. Although the bulk of the xylem in an old stem of *Microcycas* consists of pitted tracheids (fig. 6), the metaxylem and a considerable width of secondary xylem show only scalariform thickenings; at first fibrous, but farther out provided with a border to each mesh of the ladder, and followed by the well known transitional elements (fig. 7), which soon merge into tracheids whose radial walls are more or less thickly covered with bordered pits. The scalariform zone extends in some cases as many as 40 rows of tracheids, and this region may be regarded as the juvenile stage of the xylem. In this connection it is interesting to note that genera such as *Stangeria* and *Zamia*, which have a tuberous habit, appear never to get beyond this juvenile stage, for their secondary xylem consists entirely of scalariform tracheids, mixed of course with parenchyma. As may be seen in figs. 16 and 17, the scalariform tracheids of *Microcycas* show the bars on all sides, while the pitted tracheids have the pits restricted almost entirely to the radial faces, as is the case in *Dioon* and *Cycas* (5, 12).

The long gradual sequence of narrow spiral, narrow scalariform, wide scalariform, scalariform with border, transitional and finally pitted tracheids, furnishes a remarkable illustration of the recapitulation doctrine, and points to the low position of the cycads among the seed plants. The series is almost as complete, although not quite so gradual, as the classic case of *Cordaiteles* (*Dadoxylon*)

Brandingii figured by SCOTT (16), PENHALLOW (13), and recently reexamined and discussed by BAILEY (1).

The growth rings which CHAMBERLAIN found so plain in the stem of *Dioon spinulosum* and *D. edule* could not be seen in the trunk of *Microcycas*. Pieces of stem which had lain for a time in iodine solution showed considerable difference in depth of staining in different layers of the xylem, but this was found to be due only to a large proportion of starch-bearing parenchyma in the deeply staining layers, and the layers were irregular and few in number. Radial sections of the wood, however, showed what may possibly correspond to growth rings, for the broad zone of scalariform tracheids previously described is more or less clearly segregated into about three layers, each made up as follows: eight or ten scalariform tracheids, then a transitional element followed by a rather plainly pitted element. After about three zones made up in this way, all of the tracheids are of the pitted or transitional type. About two zones of this sort were discernible in the material of *Dioon* kindly furnished by CHAMBERLAIN. These appearances are not sufficiently clear cut to be entirely satisfactory evidence of growth rings, but the observations seemed worthy of record.

The wood parenchyma does not appear to have any definite arrangement; isolated cells, radial groups of two or three, and tangential groups are all to be found. Like all other available storage space, these cells are filled in the living plant with the large starch grains which are characteristic of cycads. Crystals are not a conspicuous feature in the genus under consideration. The walls show numerous simple pits (fig. 6).

Rays of three sorts may be distinguished: uniseriate, multi-seriate, and fusiform. The general disposition of these is shown in fig. 3. Rays 2-4 cells thick with a height of 15-30 cells are very common. The fusiform rays may have a thickness of 15 or more cells at the middle, and contain a leaf trace. The trace in the middle of fig. 3 shows a connection with the secondary wood (CHAMBERLAIN 5, LANGDON 9). Such connections are frequent, and play an important part in the functioning of the leaf traces after the leaf with which they were originally connected has disappeared. The wood from which the photograph was made came from a region of the trunk

more than a foot below the crown, and showed bundles in the fusiform rays through their entire course, even projecting a short distance into the pith (fig. 18). This persistence and continued growth of the leaf traces, together with the connections with the secondary

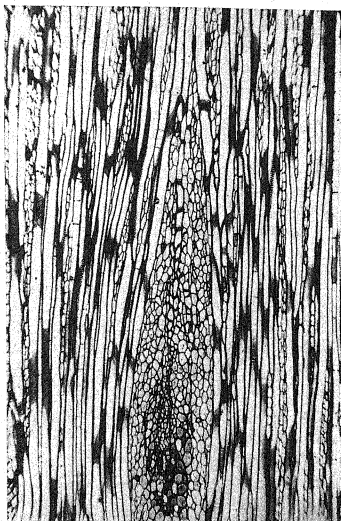


FIG. 3.—Tangential section through mature wood; part of a broad ray occupies middle region, and in it a tracheid may be seen joining the leaf trace with the secondary xylem; $\times 35$.

tracheids, indicate that the traces take on a new function when they cease to supply leaves, namely, the connecting up of the inner and outer layers of wood. In these old leaf traces the xylem is the only part which remains functional. In contrast to the pitted tracheids of the surrounding wood, the traces show only scalariform tracheids, or at most a few elements with transitional stages to the pitted con-

dition. It will be recalled that persistent leaf traces occur also in *Araucaria* and *Agathis*. The cells comprising a ray are usually two or three times as long in the radial direction as in the other dimensions, and are provided with pits which bear some resemblance to sieve plates. Such pits occur abundantly in the cells of pith, cortex, and wood parenchyma (fig. 6), and no doubt serve to facilitate storage and the utilization of stored foods.

PHLOEM.—Three kinds of elements occur in *Microcycas* as in the other genera of cycads: sieve tubes, parenchyma, and fibers. It may be seen in fig. 5 that the radial rows of phloem cells are separated by the somewhat wider cells of the rays, that the hard and soft elements of the bast are mixed without rule, and that the proportions of hard and soft elements vary in different regions. The preservation of the phloem in the material collected at Consolacion is particularly good, and has made it possible to secure satisfactory preparations of the soft bast. Like the tracheids, the sieve tubes are on a liberal scale, having a diameter of $35\ \mu$ in many cases, although some are only half that width. The sieve plates show very clearly when stained in iron-alum haematoxylin, and are shown in figs. 19 and 21. The sieve plate typical of cycads is found where two sieve tubes are in contact in the tangential plane, hence is best seen in radial sections. It differs essentially from the sieve plate of ferns on the one hand and conifers on the other. It is perhaps safer to speak of sieve areas than sieve plates, for the area covered by pores may extend for a considerable distance with slight interruptions, especially in the reproductive axis (fig. 22). On account of the width of the sieve tubes, the sieve areas are apt to be large, and their horizontal extent is greater than the vertical, but there is great irregularity in their size and shape, and they show a distinct tendency to be bunched. The pores are smaller but much more numerous than is the case in a fern such as *Pteris*. A second type of sieve plate occurs where a sieve tube comes into contact on its radial face with a fiber or parenchyma cell. As seen in fig. 19, it is a smaller and more diffuse group of pores, giving the effect of a general speckled wall, the speckles however being more or less grouped. Rather similar groups of pores occur in great numbers on the walls of the parenchyma cells, including the ray cells, in which the pores are quite conspicuous.

This porous condition is found also in the part of the ray traversing the xylem, as has already been mentioned, and is represented even in the fundamental tissue, especially of the cortex. Such pitting has been reported for the ray cells of a number of dicotyledons (10).

The form of the parenchyma cells of the phloem is clearly shown in fig. 19. They are found associated with the sieve tubes, but there appears to be nothing definite about their arrangement. Some of them contain considerable starch, while others are entirely devoid of it. STRASBURGER (18) considered that these starch-free cells function as companion cells, and this may be the case, although there is no difference in form or position between these and the regular storage cells of the phloem.

The fibers of the phloem are a conspicuous feature, on account of their great number and thick walls. In the earliest formed phloem they are relatively scarce, but in the later formed layers they are much more abundant than the elements of the soft bast. Old stems in fact show a phloem made up almost entirely of fibers in the later layers. The sparsity of fibers in the early phloem is probably to be regarded as reminiscent of the ancestral condition, for in those Cycadofilicales in which the phloem is preserved (*Heterangium tiliaeoides*, *Lyginopteris*, *Medullosa*, *Cycadoxylon Fremyi*, 15) no thick walled bast elements have been found. A peculiar feature of the fibers of *Microcycas* is the large proportion of cellulose contained in their secondary walls, while the middle lamella contains a moderate amount of lignin, as indicated by the phloroglucin test. MILLER (12) describes the fibers of *Cycas media* as suberized, but unfortunately he does not state the microchemical reaction upon which this statement is based. CHAMBERLAIN has very kindly placed at my disposal some material of the stem of *Cycas media*. In this material, as in corresponding material of *Microcycas*, the following reactions occur: the secondary thickening of the phloem fibers (1) turns violet with zinc chloriodide, (2) turns deep blue with iodine and sulphuric acid, (3) dissolves in cuprammonium. For these reasons it may safely be inferred that cellulose is the predominant substance in the thick secondary walls of these fibers.

PITH BUNDLES.—The pith of the stem usually shows no vascular bundles, although in the neighborhood of the vascular ring in older

parts of the trunk small groups of tracheids may frequently be seen, having been detached from the main mass of xylem by reason of late growth of fundamental tissue cells. Occasionally, however, there may occur a short distance inward from the protoxylem a bundle with inverse orientation. The bundle shown in fig. 20 has half a dozen scalariform and pitted tracheids, a conspicuous cambium, and a phloem made up of hard and soft bast. Whether the sporadic occurrence of such bundles should be regarded as significant is open to question, but it will be recalled that the Medullosae, generally considered to be the probable ancestors of modern cycads, had concentric bundles, while *Lyginopteris Oldhamia* frequently exhibited the feature of pith bundles with inverted structure, and such a phenomenon was excessively developed in the different species of *Cycadoxylon*. SCOTT (17) reports the occurrence of similar strands in the peduncle of *Stangeria*. The finding of bundles with inverse arrangement of tissues in *Microcycas* may add one more item to the accumulation of evidence for the medullosan origin of the cycads.

Root

Like the stem, the root contains much parenchymatous tissue, but here it is located almost wholly in the cortex. Apparently the root grows very slowly in thickness. All of the roots examined were triarch, although DORETY (7) found tetrarch structure, "with a reduction to triarch toward the tip, in some cases" in her material of the seedling. Such variations are common in other plants. The primary xylem consists of spiral and scalariform elements of the same general type as is found in the stem, but the secondary xylem is almost entirely of the pitted sort. We have here an additional argument for considering the pitted tracheid to be the ancestral type, in view of the well known conservative tendency of the root. Bast fibers have a relatively poor development. Mucilage canals are absent.

Leaf

The main features of the structure and course of the leaf trace bundles of cycads have been familiar since the classic work of METTENIUS (11), who traces in *Cycas* and *Dioon* the change from endarch to mesarch, and finally a close approach to exarch struc-

ture. Recently THIESSEN (19) has made a careful study of the cotyledonary traces of *Ceratozamia*. *Microcynas* presents a few features of interest. In the greatly swollen base of the petiole the bundles have lost the greater part of the centrifugal xylem which they possess in the cortex, and have taken on a great amount of centripetal xylem which here forms a U-shaped mass (fig. 12). In contact with each arm of the U, and also with the centrifugal elements of the primary xylem, is a well developed mass of secondary xylem, showing rays as well as tracheids. As the bundles pass outward into the petiole proper, they quickly lose all of their secondary wood, and all but a few elements of the centrifugal xylem, while the centripetal wood becomes a somewhat rounded mass constituting from here onward the bulk of the xylem. One of the bundles from the lower third of the rachis is shown in fig. 13, from which may be seen the very small amount of centrifugal xylem (restricted in this bundle to two tracheids), also the absence of secondary xylem, although several layers of the phloem have the radial arrangement. The longitudinal view (fig. 8) shows a more typical protoxylem than is found in the stem, made up of long spiral elements which have become torn by the elongation of the rachis. The centrifugal xylem is represented in this case by a single narrow tracheid with scalariform thickening, and is separated from the protoxylem by a band of parenchyma, as has been observed in other genera. The centripetal xylem presents a series of wide spiral, scalariform, and transitional elements. The pitted condition characteristic of the tracheids of the stem is scarcely reached, and the scalariform tracheid is decidedly the typical one. Comparing fig. 8 with BAILEY'S figure of the corresponding region of *Ceratozamia*, it will be seen that *Microcynas* lags behind *Ceratozamia* in this regard. The occurrence of pitted tracheids in the petiole of cycads may be taken to indicate that there were such elements in the primary wood of the forerunners of this group. This implication is in fact borne out by the fact that the primary xylem of *Medullosa* consists in part of pitted tracheids. In view of these considerations, the persistence of scalariform tracheids for some distance out in the secondary wood of *Microcynas* appears all the more remarkable, and is probably to be interpreted as the persistence of a juvenile feature which in geophilous cycads, such

as *Zamia*, lasts throughout the life of the plant. The only conspicuous feature of the phloem of the petiolar bundles is the almost total absence of the bast fibers so abundant in the stem. Many of the bundles show no fibers whatever, while other bundles containing as many as 100 phloem elements show one, two, or at most three bast fibers. This is in accord with the conservative tendency of the leaf. Each of the numerous veins which traverse each leaflet contains a bundle built on the same general plan as those of the petiole but much smaller, and surrounded by a sheath containing elements of two sorts: thick walled fibers with dense contents, and fairly thin walled empty looking cells, which certainly fit in with WORSDELL's description applying to leaflets containing numerous bundles: "inconspicuous and scarcely recognizable as transfusion tissue such as it is seen in *Cycas*" (20). The cells in question show no bordered pits nor reticulate markings, and would probably pass unnoticed if the striking condition in *Cycas* had not already been observed. In passing it may be mentioned that the leaflets of *Microcycas* generally fall off in the process of drying, in which respect they differ from the leaflets of *Zamia* and other genera.

Cone axis

The vascular tissue of this region does not form a continuous cylinder as is the case in the stem, but consists of a circular row of bundles. Unlike the leaf traces, these bundles show a liberal amount of secondary tissue, with distinct rays and a large proportion of phloem, which, however, is entirely devoid of fibers (fig. 10). In accordance with the greater food transport to the megasporangiate cone, its phloem is extremely well developed, with very numerous wide sieve tubes having the sieve areas so extensive as nearly to cover the radial walls (fig. 22), while the woody part of the bundle is more or less broken up into narrow strips separated by wide rays. The entire absence of pitted tracheids in either of the cones is to be remarked; only spiral and scalariform elements have been observed. The protoxylem elements are at the tip of each wedge-shaped mass of xylem, in sharp distinction from the condition in the leaf bundles. SCOTT (17) has described for *Stangeria* and three other genera a small amount of centripetal xylem in the bundles of the peduncle. A

search for these elements has been made in *Microcycas*, and they appear not to be nearly so well developed as in *Stangeria*, although present to the number of two or three scalariform tracheids in bundles of the stalk of the microsporangiate cone, as shown in figs. 9 and 11. These tracheids are short and interrupted, and perhaps for this reason none can be found in some of the bundles. SCOTT speaks of the shortness of similar tracheids in *Stangeria*, and attributes it to their comparatively late development. They are separated from the rest of the xylem by a few wide parenchymatous cells. None have so far been observed in the megasporangiate cone, nor in the axis of the cone itself. It is interesting to note that it was the microsporangiate cone in which SCOTT found the best showing of centripetal wood in the case of *Stangeria*. A few cases of horseshoe-shaped and of truly concentric bundles have been found in the cortex of the peduncle of the megasporangiate cone. Particularly plain cases of centripetal xylem occur in the stalk of the megasporophylls. From three to five bundles run up through this stalk, and these small bundles generally show several centripetal tracheids whose walls are provided with scalariform thickenings like those of the centrifugal portion of the xylem. The amount of centripetal xylem in these bundles appears to be as great as that figured by WORSDELL (21) in the case of *Stangeria* and *Dioon*.

Discussion

When *Microcycas* was first studied, the suggestion was made by CALDWELL that it "may be regarded as the most primitive cycad yet described" (2). The investigation of its histology ought to throw some light on this question. The concept "primitive species" has of late been reinterpreted in accordance with the doctrine of conservative organs advocated by English anatomists and emphasized by the work of JEFFREY (8). Since the various organs or regions of a plant differ in conservatism, and since one supposedly conservative organ may lag behind others of the same plant, a primitive species has come to mean one in which primitive characters predominate either in number or supposed significance. For instance, the extreme conservatism of the megasporophyll of *Cycas* might be rated more highly than the character of the vernation. A discussion

of relationships might naturally lead to a consideration of all the genera, but such is outside the scope of the present paper. We shall rather point out certain of the criteria which have been advanced for determining the position of cycad genera, and apply these criteria to *Microcycas*.

The basis upon which *Microcycas* was regarded as primitive by CALDWELL is undoubtedly the large number of sperms produced in the male gametophyte. This number exceeds even that produced by a fern antheridium. Related to this point is the exceedingly large number of archegonia, a feature regarded by CHAMBERLAIN as a highly specialized one. The embryogeny is still imperfectly known in this genus, and so furnishes no basis for comparison. The number of cotyledons was earlier (2) stated to be three to six, but DOREY (7) finds the usual number to be two, thus bringing this genus in line with the other genera. The fact that the microsporangia are scattered over the face of the sporophyll, instead of being arranged in sori, is probably to be regarded as an advanced feature. The peltate form of the megasporophyll, lacking any semblance of leaf form, would place the genus very close to *Zamia*, which is considered by CHAMBERLAIN (6) to be the most highly specialized genus in this respect.

Passing on to the vegetative organs, *Microcycas* belongs to the group of genera possessing a definite trunk. The fact that the cycads of earlier periods appear at any rate not to have had merely a subterranean stem or caudex would indicate that genera such as *Stangeria* and *Zamia* have secondarily assumed this habit, and that the treelike habit is to be regarded as ancestral. Another gross character is the marked persistence of the leaf bases, extending over many years. This may be taken to be the continuance of an early habit. Two leaf characters may stand as criteria, one being the vernation, which in *Microcycas* shows no indications of a fern ancestry. Moreover, the general habit of the leaf is less fernlike than some other genera, for instance *Stangeria*.

Coming to the internal structure of the plant, the single vascular cylinder of the stem is to be noted, in sharp contrast to the polyxylic habit of *Cycas* and *Macrozamia*. If the current view that *Medullosa*

is ancestral to the cycads be adopted, the monoxyle habit would stand as a feature of reduction. Another significant feature of *Microcycas* in common with the other arboreal genera is the pitted tracheid as the characteristic unit of the mature xylem, contrasting with the scalariform tracheid occurring exclusively in the stem wood of *Stangeria* and *Zamia*. Inasmuch as the fossil genera *Cycadoxylon*, *Lyginopteris*, *Heterangium*, and *Medullosa* show a xylem made up of pitted tracheids, this feature may be regarded as ancestral. A closely connected feature is the pronounced slowness with which the mature condition characterized by pitted tracheids is reached; it has been pointed out that a broad zone of scalariform elements precedes the mass of pitted tracheids. A comparative study of the genera from this point of view remains to be made. It is typical of cycad leaves to have the centripetal xylem strongly developed; in *Microcycas* the centrifugal elements of the primary wood are very few, and may even be absent. We see here the culmination of a movement beginning in some ancestor with a clearly mesarch leaf trace. The prevalence of scalariform and transitional elements in the primary xylem of the leaf can hardly be regarded as pointing to a primitive condition, in view of the presence of undoubted pitted tracheids in the petioles not only of *Cycas* but even of *Zamia*, a genus in which none of these elements occurs in the stem. The scarcity of centripetal xylem in the cone axis of *Microcycas*, compared with *Stangeria*, does not indicate a particularly low place in the list for the former genus. The centripetal tracheids appear to be in their last stages of disappearance, as would be appropriate to a truly advanced genus. The occurrence of scalariform and not pitted tracheids in the cone may point to a remote ancestor, but we are not in a position to contrast the other genera of cycads in this particular. The same may be said with regard to the scarcity of phloem fibers in the primary phloem of the stem and in the leaf traces, and the entire absence of these fibers in the cone axis.

Taking the various criteria into consideration, it would appear that *Microcycas* is far from the bottom of the list of genera, although it may be regarded as less specialized than such a subterranean genus as *Zamia*, to which it apparently stands close.

Summary

1. An extension of the range of *Microcycas* is noted, recording the occurrence of a station on the lowlands.
2. The trunk differs from that of *Dioon* in having conspicuous and persistent external rings composed of leaf bases separated by wider spaces occupied by scales.
3. The xylem of the stem consists typically of pitted tracheids, but there is a remarkably wide inner zone of scalariform elements representing a juvenile stage.
4. The scalariform zone shows some indication of division into a few growth rings.
5. Protoxylem is definitely present in the stem. It is endarch, and consists of narrow tracheids with close spiral and reticulate thickenings, followed by scalariform elements of increasing diameter.
6. The leaf traces are persistent, and become secondarily attached to the later formed tracheids of the woody cylinder, establishing a connection between the inner and outer wood.
7. The phloem of the different organs varies as to presence of bast fibers. A phylogenetic significance is attributed to this.
8. Pith bundles with inverse orientation occur sporadically.
9. A small amount of centripetal xylem is found in the peduncle of the microsporangiate cone.
10. A consideration of the various features leads to the conclusion that *Microcycas* is a distinctly advanced genus.

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EXPLANATION OF PLATES XVI-XVIII

PLATE XVI

FIG. 4.—Transverse section through xylem of stem; edge of one of the broad rays appears at left; $\times 110$.

FIG. 5.—Transverse section through phloem of stem; earlier formed phloem at upper side, marked by relatively few fibers; $\times 110$.

FIG. 6.—Radial section through mature xylem of stem; at left a row of parenchyma cells, showing many small pits; $\times 120$.

FIG. 7.—Radial section through transition region of xylem; stray starch grain appears in one of the tracheids; parenchyma at right; $\times 170$.

FIG. 8.—Longitudinal section through xylem of bundle of petiole; at right a scalariform element belonging to centrifugal wood; $\times 230$.

FIG. 9.—Longitudinal section through xylem of bundle from peduncle of microsporangiata cone; at left a few centripetal tracheids, followed by protoxylem; $\times 140$.

PLATE XVII

FIG. 10.—Transverse section through bundle of peduncle of microsporangiate cone; $\times 75$.

FIG. 11.—Part of bundle shown in fig. 10; near large tannin-filled cell are two tracheids belonging to centripetal wood; $\times 115$.

FIG. 12.—Transverse section through bundle in swollen basal region of petiole; U-shaped primary xylem in contact with secondary xylem; $\times 115$.

FIG. 13.—Transverse section through bundle of lower third of rachis; secondary wood has disappeared, and only a few centrifugal tracheids present; $\times 120$.

FIG. 14.—Transverse section through tip of xylem wedge next to pith of stem, showing position and appearance of protoxylem; $\times 130$.

FIG. 15.—Similar view, but through older part of stem, showing separation of tip region of xylem through growth of fundamental tissue cells; $\times 70$.

PLATE XVIII

FIG. 16.—Tangential section through inner region of xylem of stem; tracheids have scalariform thickenings on all faces; $\times 85$.

FIG. 17.—Tangential section through outer region of xylem of stem; tracheids have bordered pits on radial walls; rays and parenchyma also shown; $\times 85$.

FIG. 18.—Transverse section through inner region of xylem of stem, showing in one ray a bundle which projects into pith; $\times 35$.

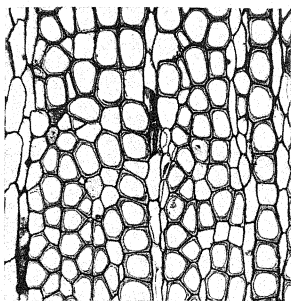
FIG. 19.—Radial section through phloem of stem; at left are sieve plates of type occurring where two sieve tubes are in contact; at right is type of sieve plate occurring where a sieve tube touches a parenchyma cell; $\times 200$.

FIG. 20.—Transverse section through pith bundle having inverse orientation; $\times 40$.

FIG. 21.—Another view of sieve plates found in stem; $\times 200$.

FIG. 22.—Longitudinal section through phloem of bundle of peduncle of microsporangiate cone; the wide sieve tube shows extensive sieve areas; $\times 120$.

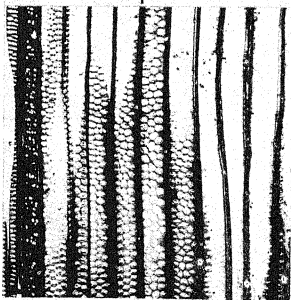
FIG. 23.—Tangential section through protoxylem of stem, showing close spiral thickenings characteristic of tracheids in this region; $\times 275$.



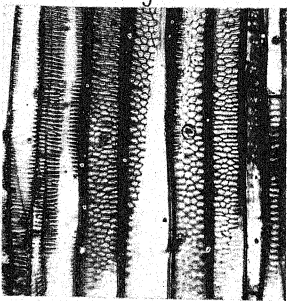
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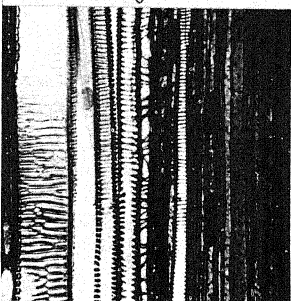
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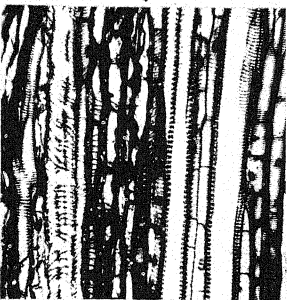
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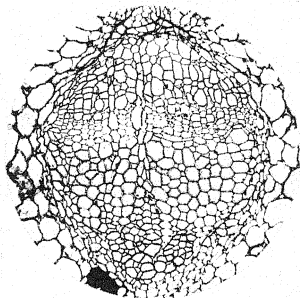
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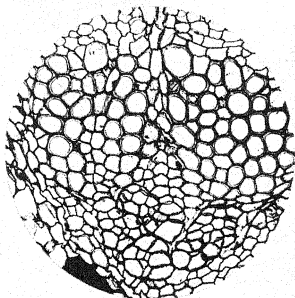
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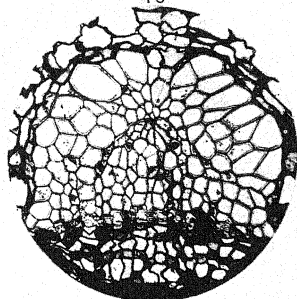
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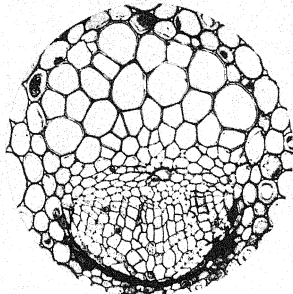
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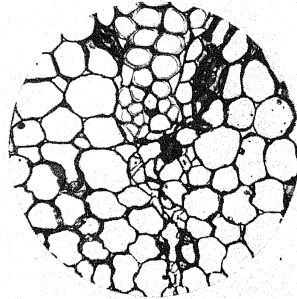
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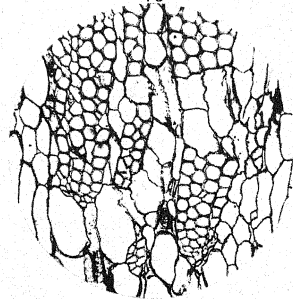
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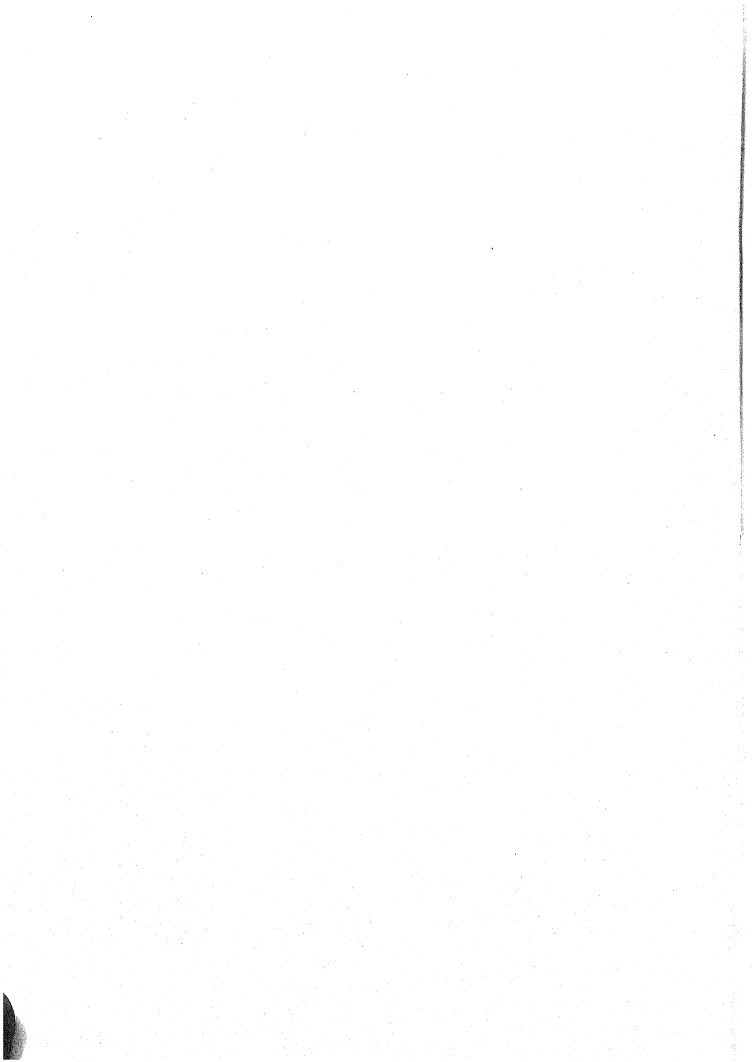
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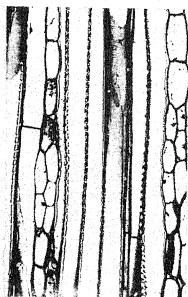


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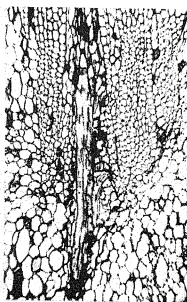




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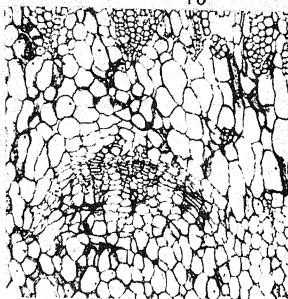
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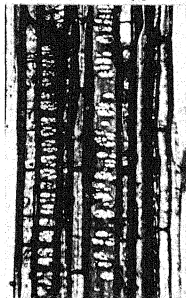
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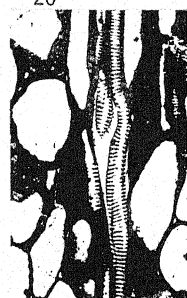
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AN ECOLOGICAL STUDY IN UTAH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 356

P. ALICE EVANS

(WITH FOURTEEN FIGURES)

Introduction

The region described in this paper comprises the greater portion of Salt Lake County, Utah, excluding in the west the foothills and mountains of the Oquirrh Range, and in the east three canyons (Mill Creek, Parleys, and Red Butte) of the Wasatch Range. This region offers so much topographical, geographical, geological, and botanical variety that it appeared to present unusual opportunities for a study in plant ecology.

Situated on the eastern boundary of the Great Basin, having within its confines Great Salt Lake, and provided by reason of the Wasatch fault with high mountain ridges down which mountain streams (aided in some instances by glacial action) have cut steep canyons, there is found here within a comparatively small area a wide variation in altitude, soil conditions, precipitation, and climate.

The region studied can be divided into two very contrasting portions: (1) the Salt Lake Valley, extending westward from the foothills of the Wasatch Range to the southwestern shores of Great Salt Lake; and (2) the mountainous area from the foothills up four of the main canyons to the crest of the Wasatch Range, which forms the eastern limits of the county. Both of these areas are bounded on the north and south by the county lines. The map of Salt Lake County (fig. 1) shows the area studied.

The valley and mountain bases were at one time part of Lake Bonneville, which existed in the Great Basin area (3) during the Quaternary period (fig. 2). The shore levels of Lake Bonneville extended 1000 feet above the level of its remnant, the present Great Salt Lake. The soils in the valley are those typical of water deposition, and are of considerable depth. Just north of the county, wells drilled 2000 feet deep still encounter this lacustrine material (9).

The various hypotheses concerning the nature of Lake Bonneville mostly contend that it was alternately fresh and alkaline during its periods of oscillations. The shore levels which clearly show on the slopes of the adjacent mountains are proof enough of this. The fine character and the variation in the amounts of deposition caused

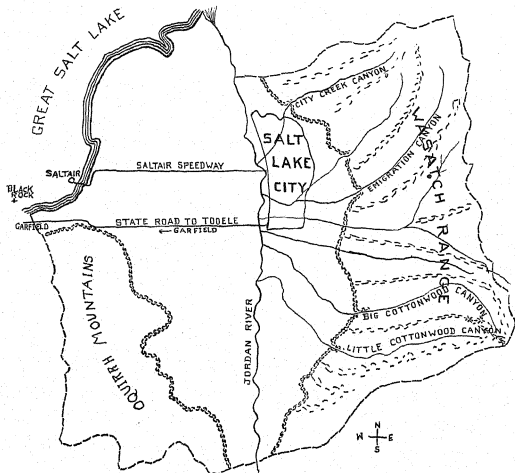


FIG. 1.—Salt Lake County, Utah, showing area studied

GILBERT (6) to believe that the chemical content of the water varied, causing during the different levels of the lake different degrees of soil and salt precipitation. As desiccation took place the chemical constituents increased, and their ultimate deposition and concentration resulted in the alkali lands and the present Salt Lake. KEYES (7) thinks that the fine depositions are of an aeolian nature; that is, the dust carried in from the arid southwest would settle on the lake surface and gradually be deposited at the bottom, there to remain. Desiccation would account for the present alkalinity of lands and water.

Both prior to and synchronous with the periodic oscillations of Lake Bonneville were periods of glaciation in the high Wasatch Mountains. The morainic deposits which occur along the sides and near the mouths of some of the glaciated canyons give evidence that there were at least two glacial epochs here. Portions of the outer morainic drifts are buried under beds of sand and gravel. The

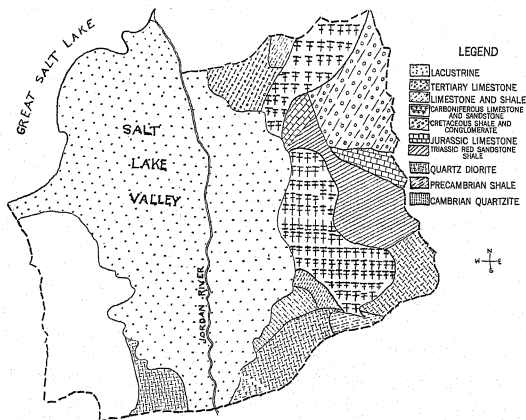


FIG. 2.—Geologic map of Salt Lake County, adapted from U.S. Geologic Map IV in *The ore deposits of Utah* by BUTLER, LAUGHLIN, and others. Professional Paper III of U.S. Geol. Survey. 1920.

burial of the earlier drift by lacustrine material indicates clearly that they are at least pre-Bonneville in age. The older deposits have disintegrated so much more than the later deposits, that it is supposed that the interglacial epoch must have been longer than the postglacial epoch. ATWOOD (1) states:

Furthermore, the outer moraines above the Bonneville shore have suffered much erosion. They are softened in contour and somewhat dissected, whereas the inner moraines are fresh in topographic appearance as well as in the condition of their material.

This also shows that the ice of the earlier epoch was more extensive than that of the later.

Since Quaternary times the temperature has increased and humidity decreased, causing the lake and glaciers nearly to disappear. Most of the canyons of the region of the Wasatch Range have large streams, which since the retreat of the lake and ice have eroded gorges in the morainic drifts and through the lake shore terraces, and formed in some instances rather large deltas at their mouths.

The purpose of the investigation recorded in this paper was to study the narrow strip of the Great Basin area included in Salt Lake County, Utah, and the adjacent canyons in the western Wasatch Range as to geological formation, and also as to the vegetational cover, including the effect thereon of climatic and other local modifying factors. The canyons selected for this study were four, two of which are representative of the main glaciated area of this region, namely, Big Cottonwood and Little Cottonwood canyons, while the others, Emigration and City Creek canyons, are unglaciated.

The discussion of this study will be made in the following order: (1) the lowland or Salt Lake Valley; (2) the unglaciated canyons; and (3) the glaciated canyons.

Salt Lake Valley

The valley as shown on the maps is divided by the Jordan River into an east and a west portion. The Jordan River rises in Utah Lake, a body of fresh water about 10 miles south of Salt Lake County, flows north through the county, and empties into Great Salt Lake.

The soil of the valley has had a common origin, namely, that of a lacustrine deposition, but there is now a great difference in chemical composition between the soil on the west side of the Jordan River and that on the east side. This is doubtless due to the fact that the fertile soil on the eastern side consists largely of eroded material from the mountains, which has been prevented from reaching beyond the Jordan River because the river flows parallel to the base of the mountains. The examination of the valley section, therefore, has naturally been divided into two very different areas, based

on soil composition: (1) the land west of the Jordan River extending to Great Salt Lake, which area, excepting a narrow strip of land about 2 miles in width adjoining the river and some land south of the Garfield Road near the base of the Oquirrh Range, is of an alkaline nature and decidedly unfertile (11); and (2) the land east of the Jordan River extending to the foothills of the Wasatch Range, which is very fertile if water is available.

Logically the beginning of the study is in the western area, on the shores of Great Salt Lake. Fig. 3 shows the most western point of study in the region of Black Rock, an isolated granite post standing in the water about 70 feet from the railroad, and used only as a landmark. Here the shore of the lake has been greatly modified, due to the construction of the Western Pacific Railroad. Wooden piling driven along the margin of the lake is filled with rocks, thus separating the main body of water from the marshes that lie at the base of the limestone cliffs of the Oquirrh Range, which here reaches its northern limit. The study here dealt with the vegetation around the marshes and on the tops of the cliffs, and is recorded as follows:†

MARSHES

Alopecurus fulvus
Atriplex confertifolia
Abronia salsa
Distichlis spicata
Iva axillaris
Phragmites communis
Ranunculus Cymbalaria
Salix fluviatilis
Sesuvium sessile
Sarcobatus vermiculatus
Salsola pestifer
Trifolium longipes

VEGETATION ON DRY CLIFFS

Asclepias speciosa
Ascerates viridiflora linearis
Achillea millefolium
Atriplex confertifolia
Atriplex canescens
Bromus tectorum
Monothrix Stansburijii
Malvastrum coccineum
Sarcobatus vermiculatus
Salsola pestifer
Eurotia lanata

About one mile east of Black Rock the railroad turns inland, leaving the shore line undisturbed. Here is a bare sand beach, irregular in width due to the oscillations of the lake level, which is stationary only when rainfall and inflow equal evaporation. This is not often the case, as will be shown in the discussion on the climatic

† The names follow the nomenclature of COULTER and NELSON (4); in a few instances they were obliged to be taken from GARRETT (5) and RYDBERG (10).

conditions of the region. Also wind storms cause variation in the advance of water, which is marked by drift wood and dead vegetation. The plant that here ventures nearest the water's edge is *Distichlis spicata*. Associated with it, near the zonal margin away from the lake, are to be found *Juncus balticus* and *Alopecurus fulvus*. Just behind this salt grass area is a raised beach which might be termed a foredune. On this raised portion are to be found such plants as *Anogra cinerea*, *Asclepias speciosa*, *Sporobolus airoides*, *Chrysothamnus graveolens glabrator*, *Iva axillaris*, and *Kochia vestita*.



FIG. 3.—Shore of Great Salt Lake and Black Rock at Garfield, Utah

Just south of the foredune are moist depressions and brackish marshes, the banks of which are inhabited by *Typha latifolia*, *Juncus balticus*, *Phragmites communis*, *Salix fluviatilis*, *Atriplex confertifolia*, and *Distichlis spicata*, which still grows here where not crowded out by other vegetation.

These three vegetational zones paralleling the lake shore line continue eastward only a short distance. They are again interfered with by a slag dump from the Garfield smelter, which is situated on the north-facing foothills of the Oquirrh Range. This disturbed area was not studied, it being plantless in the main.

The study was taken up again on the eastern shore of the lake near the Saltair Resort (fig. 1). Here there is a large plantless beach which is of a very miry nature, covered by a heavy deposit of alkali salts, algae, and larva cases of *Ephedra* fly. This is a most unpleasant beach to look upon, and if the material is disturbed the resulting

odor is equally unpleasant. As the substratum becomes firmer it is occupied by a scant stand of *Allenrolfia occidentalis*, *Salicornia utahensis*, and *S. rubra*, which are the most salt-tolerant plants of this region. Just east of this area is a less alkaline zone, which, with the exception of the commercial salt fields that occupy a considerable area, is covered by a sparse vegetation, the dominant plants being *Atriplex confertifolia*, *Distichlis spicata*, *Chenopodium salinum*, *C. rubrum*, *Sarcobatus vermiculatus*, *Salsola pestifer*, and *Kochia vestita*. This type of vegetation, excepting in the vicinity of the salt fields, covers the greater portion of the lower and level areas west of the Jordan River and north of the Garfield automobile road, comprising in all about 125 square miles. On the higher areas are to be found *Kochia vestita*, *Grayia spinosa*, *Poa Sandbergii*, and *Sphaerostigma pubens*; while on the crest of these raised areas are *Artemisia tridentata*, *Gutierrezia Sarothri*, *Erodium cicutarium*, and *Bromus tectorum*.

The land adjacent to the river and south of the Garfield road is in the main higher than that just described, and the alkali, if it was ever present, has been drained away. In the natural condition these higher benches are covered by a growth of *Artemisia tridentata*, *Chrysothamnus nauseosus albicaulis*, and various annuals and grasses. Along the river are also found *Salix fluviatilis*, *Rosa Nutkana*, *Apocynum* sp., *Acer Negundo*, and tall herbaceous plants such as *Rumex crispus*, *Chenopodium album*, and *Urtica* sp.

Most of this river bench and south portion of the land west of the Jordan River is of fair agricultural value, and has successfully been cultivated for several decades. The crops in the main are alfalfa and sugar beets, with some garden truck. There are also a few orchards.

The northern and alkali region to be of any agricultural value must be drained. It is an excess of sodium carbonate or black alkali which is the principal obstacle here. Even converting this by the use of gypsum into white alkali would still leave an excess of salts in the soil, so that drainage seems to be the only feasible solution of the problem. During the last few years attempts have been made to reclaim portions of the alkali land by the construction of drainage canals. About 90 square miles are said to be capable of reclamation in this manner.

The land east of the Jordan is about 4450 feet above sea level, and is 230 feet higher than the shore of Great Salt Lake. The gradient is rather steep and merges into the foothills of the Wasatch Range. Where water from the Jordan River and from the Canyon streams has been available for irrigation, crop cultivation has been carried on. The land is very fertile, and the climate favorable to such crops as peaches, apricots, cherries, and apples; small fruits in abundance, such as currants, gooseberries, raspberries, and strawberries, and many kinds of melons are also grown.

The large streams from the canyons have produced rather large deltas, especially City Creek and Big and Little Cottonwood Canyon streams. Salt Lake City occupies all of the City Creek delta and the adjacent benchland to the north and east. The other deltas are occupied mainly by farm land. The lowlands between are mostly meadow and are ideal for dairy farms. The virginal stand of vegetation has long since disappeared from this side of the valley, due to the extensive agriculture on the lower lands and the overgrazing of the benchlands by sheep and cattle. Even as early as 1875, during the Powell Geological Survey, GILBERT (6) observes:

In the virgin condition most lowland valleys and all of the upland valleys were covered by grass and other herbaceous vegetation. These have been eaten off by the herds of the white man and in their place has sprung up a sparse growth of low bushes between which the ground is bare.

However, although this area has been overgrazed and also burned over frequently and otherwise modified by man, there still remains a great differentiation between the lowland and benchland flora. Individual owners have protected certain areas, so that we can get some idea of the probable original vegetational cover of the east valley under these divisions: (1) the lowlands which are of three types (marshlands, drylands, and the vegetation along the stream banks); (2) the benchlands, including protected and unprotected areas. The vegetation is listed under these headings in the table.

LOWLANDS

LOW MARSHLANDS

Acer Negundo
Populus angustifolia
Salix Scouleriana

LOW DRYLANDS (TREELESS AREAS)

Purshia tridentata
Chrysothamnus nauseosus
Grindelia squarrosa

LOWLANDS—Continued

LOW MARSHLANDS

Typha latifolia
Sagittaria arifolia
Equisetum hiemale
Equisetum arvense
Scirpus lacustris
Juncus balticus
Sium cicutaefolium
Camassia esculenta
Brodiaea Douglasii
Valeriana ceratophylla
Viola Howellii
Ranunculus Macounii
R. sceleratus eremogenes
Onagra strigosa
Sisyrinchium angustifolium
Rumex venosus
Mimulus Langsdorfii

LOW DRYLANDS (TREELESS AREAS)

Malva rotundifolia
Phlox longifolia
Erodium cicutarium
Linum Lewisii
Bromus tectorum
Calochortus Nuttallii
Delphinium Nelsonii
Lepidium perfoliatum
Capsella Bursa-pastoris
Draba caroliniana
Glycyrrhiza lepidota
Viola venosa
Viola Beckwithii
Convolvulus arvensis
Phacelia linearis
Lappula subdecumbens

STREAM VEGETATION IN VALLEY

Populus angustifolia
Salix lutea
Salix amygdaloides

Rhus trilobata
Rosa Nutkana
Acer Negundo

BENCHLANDS

 PROTECTED AREAS (MAINLY SHRUBS
 WITH SPARSE UNDERGROWTH OF
 GRASS AND ANNUALS)

Purshia tridentata
Chrysothamnus graveolens
Artemisia tridentata
Quercus Gambellii
Erodium cicutarium
Bromus tectorum
Allium acuminatum
Malvastrum coccineum
Astragalus utahensis
Astragalus cibarius

UNPROTECTED AREAS

Achillea millefolium
Astragalus utahensis
Grindelia squarrosa
Wyethia amplexicaulus
Balsamorhiza sagittata
Taraxacum officinale
Lithospermum angustifolium
Eriogonum ovalifolium
Lappula subdecumbens
Phlox longifolia
Onagra pallida
Onagra marginata
Aulospermum longipes
Opuntia polyacantha
Viola adunca
Viola venosa

Unglaciaded canyons

CITY CREEK CANYON

Northeast of Salt Lake City is City Creek Canyon, a V-shaped canyon typical of stream erosion, running in a southwesterly direction. The substratum is mainly of limestone with now and then an exposure of trachytic tuffs and breccias, indicating surface volcanic action in the lower part of the canyon (9). Near the head and on the



FIG. 4.—Shrubs growing in depressions on foothills; photograph by SEVILLE FLOWERS.

higher mountain faces are to be found conglomerate and quartzite formations. By comparing figs. 1 and 2 one can see the geologic formations through which the canyon has been eroded.

The vegetation in this canyon has been greatly modified by overgrazing and fire. There are to be found all gradations in succession, from the grass and herb covered foothills through the xerophytic scrub formations to the spruce and aspen forest at the head of the canyon. There is a rise of about 3300 feet from the mouth of the canyon, which is 4450 feet above sea level, to the head of the canyon which is 7800 feet high. In the bottom of the canyon is a rather large stream, one of the main sources of Salt Lake City's

water supply. Along the stream is to be found a vegetation somewhat mesophytic in nature. In this, as in all the canyons studied, the character of the undergrowth changes remarkably with the seasons. In the spring there is a great abundance of ephemerals such as *Claytonia lanceolata*, *Fritillaria pudica*, *F. atropurpurea*, and *Erythronium grandiflorum*; and in the summer and autumn the more xerophytic Compositae and Malvaceae are dominant, with such plants as *Solidago canadensis*, *Senecio serra*, *Helianthus annuus*, *Malvastrum coccineum*, *Malvastrum coccineum dissectum*, and *Sphaeralcea rivularis*.

On the foothills near the mouth of the canyon, as on the unprotected benchlands of the valley, only shortlived grasses and herbaceous plants can grow. The foothills and lower ridges are burned over annually, making it very difficult for a higher type of vegetation to get started. Such areas entirely free from trees and shrubs are found on the ridges in fig. 4. Nearer the head of the canyon the same conditions still exist on the ridges; but in the depressions, especially on the north-facing slope, shrubs are starting to come in, as is shown in the figure. The isolated groups of shrubs in the depressions start as an *Artemisia tridentata* and *Rhus trilobata* association. Then *Quercus Gambellii* comes in, and the *Artemisia* disappears so that most of the patches are *Quercus-Rhus* associations with some *Ribes aureum*. In the higher patches (fig. 4) *Juniperus utahensis* comes in. The undergrowth in these oak patches is made up of such plants as *Mertensia brevistyla*, *Allium acuminatum*, *Phacelia linearis*, *Hydrophyllum occidentale*, *H. capitatum*, *Erysimum asperum*, and *Berberis repens*.

Higher in the canyon the grass cover is succeeded by an *Artemisia-Chrysothamnus* association. This in turn is succeeded by a *Ceanothus velutinus* and a *Cercocarpus ledifolius* association, with *Juniperus utahensis*, *J. scopulorum*, *Sorbus scopulina*, and *Prunus melanocarpa* coming in on the higher and north-facing slopes. Among the last named plants is also to be found *Populus tremuloides*, which soon dominates. Coming in under its shade are to be found *Picea pungens* and *Abies concolor*, which make up the greater stand of vegetation on the north-facing slope of the canyon. Figs. 5 and 6 show the contrast at the head of the canyon between the south-facing

slope, with a mere sprinkling of conifers, and the north-facing slope, with a much denser stand of conifers mingled with *Populus tremuloides*.

The succession of vegetation along the stream is more gradual, due in all probability to the more mesophytic conditions. The following plant lists illustrate briefly the vegetational succession on both south and north-facing slopes and along the stream, culminating at the head of City Creek Canyon in the scant stand of the forest of spruce and aspen.

VEGETATION OF CITY CREEK CANYON

FOREST OF SPRUCE AND ASPEN

Picea pungens

Abies concolor

Populus tremuloides

UNDERGROWTH SHRUBS

Shepherdia argentea

Prunus melanocarpa

Amelanchier alnifolia

Physocarpus malvaceus

Sorbus scopulina

Sambucus microbotrys

Pachystima Myrsinites

HERBACEOUS UNDERGROWTH

Heuchera parvifolia utahensis

Dicentra uniflora

Actaea arguta

Thalictrum Fendleri

Pyrola secunda (very abundant)

Pteridium mucronata

SOUTH-FACING SLOPE

Juniperus scopulorum

Cercocarpus ledifolius

Ceanothus velutina

Cowania mexicana

Purshia tridentata

Rubus strigosus

Artemisia tridentata

Chrysothamnus nauseosus albicaulis

NORTH-FACING SLOPE

Populus tremuloides

Prunus melanocarpa

Sorbus scopulina

Juniperus scopulorum

Pinus flexilis

Pseudotsuga mucronata

Ceanothus velutinus

Pachystima Myrsinites

Physocarpus malvaceus

Rubus parviflorus

Amelanchier alnifolia

Acer grandidentatum

Quercus Gambellii

Acer Negundo

Sambucus glauca

Artemisia tridentata

Purshia tridentata

BANKS OF STREAM

Salix Fendleriana

Salix amygdaloides

Acer glabrum tripartitum

Betula fontinalis utahensis

Alnus tenuifolia

Cornus stolonifera

Prunus melanocarpa

VEGETATION OF CITY CREEK CANYON—*Continued*

BANKS OF STREAM

Rubus parviflorus
Crataegus rivularis
Sambucus glauca
Rosa Woodsii
Rosa Nutkana
Rhus Rydbergii
Salix fluviatilis
Acer grandidentatum
Acer Negundo
Populus angustifolia
Populus tremuloides
Pinus flexilis
Picea pungens
Abies concolor

NORTH-FACING SLOPE

Chrysothamnus nauseosus
Gutierrezia Sarothrae
Agropyron spicatum*
Bouteloua gracilis*
Koeleria cristata*

* These grasses are also common in all of the foothills of the valley.

An attempt was made to determine the succession in the vegetation from the valley at an altitude of 4450 feet to the canyon head at an altitude of 7800 feet. The results secured were not very positive, except that the south-facing slope being more xerophytic, the herbaceous and grass vegetation of the foothills extends farther up into the canyon on the south than it does on the north face, hence the variety in plant forms is greater on the north face, until the spruce forest is reached where conditions are similar on both faces.

Near the head of City Creek Canyon are two small lakes that help to feed the stream that flows to the city and valley below. In early April and May these lakes are fairly large; fig. 5 shows one of these lakes in April while the water was still high. This figure also shows the contrast between the vegetation of the south-facing slope with its sprinkling of conifers, and that on the north-facing slope with its denser stand of conifers. During the warmer months of the summer these lakes become almost dry, and in very hot and rainless seasons they are completely so. As the water recedes, willows and herbs come in to occupy the land. *Salix Fendleriana* and *S. lutea* seem to be the dominating willows, while *Mentha spicata*, *M. canadensis*, *Urtica Breweri*, and *Mimulus Langsdorffii* are the dominant herbs. Fig. 6 shows the lake taken the same year, but in September. If it were not for the fact that the surrounding mountains are the

same it would be difficult to believe that this is the same lake as shown in fig. 5. In fig. 6 the vegetational growth appears to be rather dense; this is because some of the herbs have grown to be almost as tall as the willows. The woody growth is really not very thick, the lake bed being covered mainly by herbs and reeds.

The reasons for the disappearance of the water here are probably two: (1) the evaporation in this region is rather great, as is shown in the notes on climatic conditions which follow this discus-

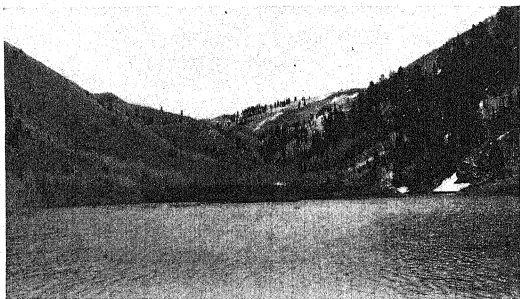


FIG. 5.—Lake at head of City Creek Canyon, April, 1923; wooded side is north face of canyon; photograph by SEVILLE FLOWERS.

sion; and (2) since the substratum is mainly of limestone it may be supposed that the seepage is considerable. This idea is further supported by the fact that lower in the canyon, underground streams come to the surface as large flowing springs.

EMIGRATION CANYON

The other unglaciated V-shaped canyon, Emigration Canyon, has its head in the same mountains of Cretaceous conglomerate rock as does City Creek Canyon. It differs from the latter, however, in that its direction is south by west, and there are scattered along the ridges outcroppings of Triassic red sandstone. The more southern direction exposes the slopes of both sides of the canyon to about the

same amount of sunlight, consequently there is shown in the vegetation on each side practically the same degree of xeromorphism. *Populus tremuloides*, *Abies concolor*, *Pseudotsuga mucronata*, and *Picea pungens*, however, come in at a somewhat lower altitude on the southeast-facing slope than on the southwest-facing slope. Other conditions seeming to be equal, this would probably indicate that the afternoon sunlight is somewhat more desiccating than the morning sun.



FIG. 6.—Same lake as in fig. 5, taken in September, 1923; photograph by SEVILLE FLOWERS.

The lower foothills and sides of the canyon near its mouth from an altitude of about 5000 to 5800 feet are covered with *Artemisia tridentata*. Below that altitude and down into the valley are grasses and herbs typical of the burned-over areas of the region.

On all the higher ridges of the canyon at an altitude of 7800 feet are to be found *Cercocarpus ledifolius* and *C. intricatus*. Below the *Cercocarpus* ridges, extending down the mountain sides into the canyon, is a wide band of *Ceanothus velutinus*, which merges into *Juniperus scopulorum* and *Quercus Gambellii*.

The next zone is a mixture of *Acer grandidentatum*, *Cornus stolonifera*, *Sorbus scopulina*, *Prunus melanocarpa*, *Amelanchier alnifolia*, and occasional *Pseudotsuga mucronata* and *Abies concolor*. This mixed vegetational zone extends down to the stream in the bottom

of the canyon, where it merges into the typical stream bank vegetation similar to that found in City Creek Canyon.

In the tributary gorges on either side of the canyon are found stands of *Populus tremuloides*, *Abies concolor*, *Pseudotsuga mucronata*, and *Picea pungens*, with a mesophytic undergrowth. The type of undergrowth found here consists of such plants as *Thalictrum Fendleri*, *Actaea arguta*, *Aquilegia flavescens*, *Claytonia lanceolata*, *Stellaria longipes*, *Pyrola secunda*, *Mertensia longifolia*, *Hydrophyllum capitatum*, *Arnica cordifolia*, *Erythronium grandiflorum parviflorum*, *Disporum trachycarpum*, *Smilacina amplexicaulis*, *S. sessilifolia*, *Pteridium aquilinum*, etc.

Glaciated canyons

The broad, open, basin-like heads, the U-shaped valley sections, and the tributary hanging valleys with their lakes are evidence of the work of the Pleistocene glaciers in the Big Cottonwood and Little Cottonwood canyons.

LITTLE COTTONWOOD CANYON

This, the smaller of the two canyons studied, is a beautifully U-shaped canyon. It is 12 miles long and contained the largest glacier of the region. In some places the striae on the rocks indicate that the ice was over 1000 feet thick, and the morainal deposits show that the glacier extended down into Salt Lake Valley below the mouth of the canyon (1). The main stream bed has a general westward direction, but turns at the mouth of the canyon toward the northwest.

The chief regions of study were the main catchment basin at the head of the canyon and the region between the smooth granitic walls and the canyon mouth. There are a number of tributary valleys which have interesting formations, but no attempt was made to study them.

The evident geologic formations and their respective plant coverings from the mouth of the canyon to the head appear in the following order. Near the mouth is a collection of drift material made up almost entirely of granitic and Archean rock. The vegetation is composed of the xerophytes typical of the foothills in this region, such as the *Quercus-Rhus* and the *Artemisia-Chrysothamnus* associa-

tions, and the grass-herb association leading into the valley. Passing on up the canyon the white granitic rock becomes dominant, and for six or seven miles upstream forms its very smooth and steep walls. Here the slopes are too steep to allow any but crevice vegetation to grow, and are so smooth and precipitous as to be inaccessible for close study. In the nearer crevices the tree forms, such as *Pseudotsuga mucronata* and *Pinus flexilis*, and some of the shrubs such as *Rubus strigosus gracillipes* and *Ribes cereum*, which are typical of rock crevice vegetation in this region, could be recognized. As the granite diminishes, the Paleozoic sedimentary rocks become more abundant. The main catchment basin is almost entirely of sedimentary rock. The rocks of the slopes of this basin show the erosive effect of ice to an astounding degree. ARWOOD states:

Every conceivable thing that the debris-laden ice might do in passing over a rock is illustrated: Chattermarks, polished surfaces, deep groves . . . and at one place six sets of striae indicating as many directions of ice-movement on a single surface.

The only lake in this main cirque, Mountain Lake, locally named Lake Minnie, is on the north-facing slope in a rock basin of red quartzite at an altitude of 9850 feet. The geologic map (fig. 7) shows the position of all the glacial lakes studied, of which Mountain Lake is one. Fig. 8 shows the setting and the type of vegetation which surrounds this lake. The water is not more than 8 or 9 feet deep and contains no visible form of plant life. There is also lacking a shore vegetation. Scattered here and there about the lake, as elsewhere on the north-facing slope, are clumps of small trees such as *Juniperus communis* and *Pseudotsuga mucronata*, and the shrubs *Ribes lentum*, *Ribes viscosissimum*, and *Jamesia americana*. Just above the lake is a rock mountain with a talus slope of shale. These rocks have no covering of vegetation, due probably to the fact that they are normally covered by snowdrifts the entire year, and the plants have not had a chance to get up so high in one dry season. Fig. 9 shows the rock formation just below Mountain Lake. Here the rocks are very smooth, and plants such as *Selaginella rupestris*, *S. densa*, and *Phlox canescens* are abundant. The trees are mainly *Pinus flexilis*, *Pseudotsuga mucronata*, and *Juniperus communis*, and the shrub *Ribes lentum* is fairly prevalent.

The south-facing slope of the cirque does not have such wide expanses of smooth rock. The slopes are more eroded and scarred by shallow gullies. The vegetation is scattered, and in patches,

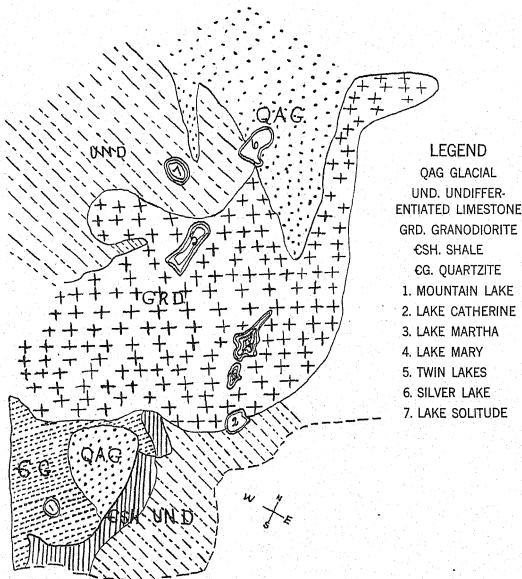


FIG. 7.—Geologic map of the Cottonwood Divide, adapted from U.S. Geologic maps in *The ore deposits of Utah* by BUTLER, LAUGHLIN, and others. Professional Paper III of U.S. Geol. Survey. 1920.

in which are found *Pinus flexilis*, *Pseudotsuga mucronata*, and *Juniperus communis*. The shrubs are *Ribes lentum* and *R. viscosissimum*. The only herbaceous plants found here are *Fraseria speciosa*, *Lupinus floribundus*, and in some of the shaded rock crevices *Primula*



FIG. 8.—Mountain Lake, Little Cottonwood Canyon

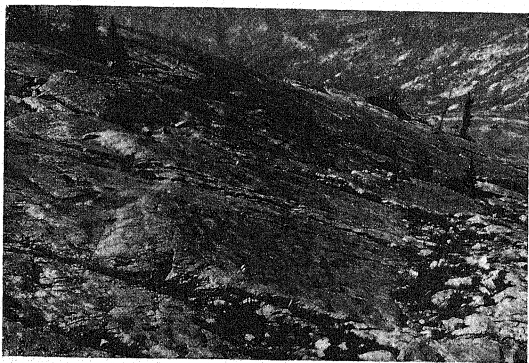


FIG. 9.—Glaciated rocks below Mountain Lake, Little Cottonwood Canyon

Parryi and *Woodsia oregana*. In crevices exposed to the sun *Pellaea densa* and *P. Wrightiana* are found. The scantiness in shrubs and herbaceous vegetation is due to the work of 25,000 head of sheep which were allowed to browse here for about a week. The plants named were those left, seemingly unpalatable to the sheep. They had eaten all spineless shrubs; the willows were stripped of all foliage, and in some instances were cropped close to the ground. Most of the spaces between the clumps of evergreens were entirely

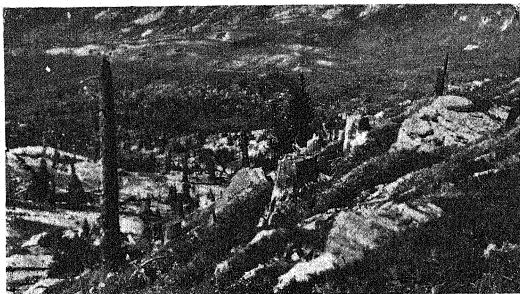


FIG. 10.—Main catchment basin in Little Cottonwood Canyon, showing effects of over-cutting and over-grazing.

bare of vegetation. This is a most deplorable condition, since it leaves the very thin layer of soil exposed to all eroding factors such as the strong westerly winds and the rainfall which is usually very heavy. This in turn naturally results in very rapid run-off, perhaps of almost "cloudburst" proportions, causing great damage lower down the canyon. These factors have already transported much of the soil elsewhere, leaving only bare rock surfaces on which it is most difficult for any plant to live. There are some lichens, but they are not very abundant.

In the bottom of the cirque conditions are not much better, although there are several small springs which aid vegetation to become more quickly reestablished. Here were found large areas of

pure stands of *Rudbeckia occidentalis*, an indication that this region has been overgrazed before. Another regrettable condition is illustrated in fig. 10. In pioneering days there was a great need for lumber, and since the timberlands in this region are high up and scattered here and there among the mountains, the most accessible trees were used. The trees in this basin apparently afforded good lumber material, and the entire stand, which was somewhat extensive and of rather large trees, was completely destroyed. It will be many generations before this area is reforested with a similar stand.

BIG COTTONWOOD CANYON

The ridge which forms the south-facing slopes in the main basin of Little Cottonwood Canyon also forms the north-facing slopes of the main basin of Big Cottonwood Canyon. As the name implies, this canyon is the larger of the two. It also contained Pleistocene glaciers, but they were not as large as the glaciers in the Little Cottonwood, and did not extend the full length of the canyon. It is broader, longer, and has much more tributary country than has Little Cottonwood Canyon, and consequently offers a much larger opportunity for ecological study.

The main catchment basin is a broad open valley at the head of the canyon, in which is located the mountain resort of "Brighton" or "Silver Lake." Summer homes are dotted along the surrounding slopes. Tributary to the main basin are six hanging valleys, each containing rock basin lakes. The streams that flow from these lakes into the big basin join the stream from Silver Lake, the only lake in the main basin, and together they make up Big Cottonwood Creek. This creek flows northwestward until, just before it leaves the cirque, it assumes a general westward direction, which it holds throughout its course in the comparatively narrow and rugged lower canyon.

The dominant geologic formations at the head of the canyon will be mentioned in the discussion of the different portions studied. These formations are shown in fig. 7 (3).

The highest peak in the Cottonwood divide, Mount Wolverine, a steep mountain 10,650 feet high in the Granodiorite formation, is at the head of Big Cottonwood Canyon. The north-facing slope of this mountain has very little vegetation. It is too steep to per-

mit of ascent, and consists mainly of bare, rugged rock ledges on which are to be found a few lichens. On the east and west ridges leading up to the mountain peak and on the less steep south face is to be found a typical subalpine vegetation. The trees have assumed the stunted growth forms of wind timber, the shrubs are reduced in size, and the herbaceous plants are the typical mat-formers of cold regions. The trees found just below the mountain peak are *Pinus flexilis* and *Abies concolor*. The shrubs are *Ribes saximontanum* and *Arctostaphylos Uva-ursi*. This is the only place where the latter shrub was found in the entire region studied. The matted herbaceous plants are *Phlox canescens* and *Sedum integrifolium*. About 300 feet lower on the mountain side were the herbaceous plants usually found in the vicinity of snowbanks and in damp places, such as *Primula Parryi* and *Aquilegia flavescens*. In years of normal precipitation there are usually large snow banks which remain on the ground until late in August, but 1924 being an unusually dry and hot year, the snow had disappeared by mid-July.

Still lower on the mountain side, leading down into the cirque which contains Lake Catharine, the highest glacial lake of the region, at an altitude of 9850 feet are found such plants as *Macaranthra viscosa*, *Castilleja* sp., *Lupinus* sp., *Orthocarpus Tolmiei*, and *Veratrum speciosum*, which occurs in large stands near the southeast end of the lake; at the spring and along the stream feeding the lake are found *Parnassia fimbriata* and *Geranium Richardsonii*. The dominant shrubs leading down the northeast face of the mountain to the shore of Lake Catharine are *Pachystima Myrsiniles*, *Ribes lentum*, *Sambucus microbotrys*, *Dasiophora fruticosa* (very abundant), and *Artemisia aromatica*. The trees here are *Pseudotsuga mucronata* and *Abies concolor*. Lake Catharine lies on two kinds of substratum (fig. 7). The southeastern half, on which this vegetation is found, is of undifferentiated limestone, and has accumulated a good layer of soil; while the northwestern half is on the Granodiorite formation and consists mainly of bare rock. Fig. 11 shows the type of vegetation found on the north side of the lake. With the exception of a few scrubby trees and woody plants which found a crevice in which to anchor, the rocks are bare. Fig. 11 also shows the paths

of drainage and snowslides into the lake, which are two of the causes for certain areas being devoid of vegetation. The only plant life visible in the lake consists of a few forms of algae.

Passing down over the northwest ridge of the Lake Catharine cirque, conditions become more mesophytic and more favorable to

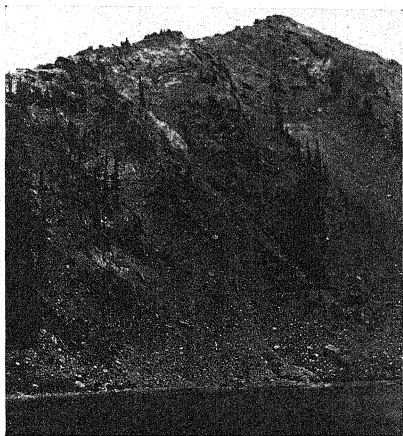


FIG. 11.—Lake Catharine in Big Cottonwood Canyon, showing course of snow slides.

plant life. The trees are in a more sheltered position and hence can obtain a more normal growth. Here are to be found such trees as *Pseudotsuga mucronata*, *Picea Engelmannii*, *Abies concolor*, and *Juniperus communis*. The shrubs are scattered in their distribution, and such forms as *Ribes lentum*, *R. coloradense*, *Sambucus microbotrys*, and *Sorbus scopulina* are found here. The rocks are kept moist by seepage water, and growing among them are the herbaceous plants *Primula Parryi*, *Aquilegia flavescens*, *Ranunculus glaberrimus*, *Saxifraga arguta*, and *Asplenium viride*.

This northwest ridge descends into a lower glacial cirque containing Lake Martha. This cirque, which is entirely in the Granodiorite formation, is 200 feet lower than Lake Catharine, and is surrounded on the east, south, and west by comparatively high and close mountains which serve as very good wind breaks. The slopes of the surrounding mountains are too steep to allow of much accumulation of soil, so that mainly a crevice vegetation is found here, with trees growing taller than those higher and more exposed. The character of the substratum of the shore and lake bottom is very different from the rocky substratum of Lake Catharine. Here there is more of an accumulation of soil, and the bottom of the lake is covered by a layer of soft mud and humus which appears to be several feet thick. The vegetational zone around Lake Martha is 15-20 feet wide, and consists of the grasses *Calamagrostis canadensis acuminatum*, *Silene rigidum*, and *Hordeum jubatum*, and reeds as an undergrowth to the willows which are here dominant plants. *Isoetes* with *Sparganium* grows out some distance into the water. One interesting feature about the line of willow growth is that one cannot make his way among the willows on account of the numerous *Abies concolor* seedlings which are coming in under the protection of their shade. These conifers are almost as tall as the willows, but yet not large enough to be noticed until one tries to pass through them. There is an interesting little rocky island in the lake, on which are growing *Pseudotsuga mucronata* and *Ribes lentum*. Fig. 12 shows this lake with its island, shore vegetation, and the northeast-facing slopes which are only scantily clothed with vegetation.

Just over the north rim of the Lake Martha cirque and about 100 feet lower in altitude is another glacial basin, in which is found Lake Mary. This basin at one time contained two lakes, but a dam was built across the northeast rim of the basin, making it into a reservoir for the water supply of Salt Lake City, thus raising the water and making the two lakes into one. The raising of the water caused the scanty shore vegetation to be destroyed. Now there is surrounding the basin a rim of bare, smooth glaciated rock, whose crevices hold mainly tree forms such as *Pseudotsuga mucronata*, *Picea Engelmannii*, and *Abies concolor*. Some *Juniperus communis* and *Pachystima Myrsinites* occur, with a few herbaceous plants grow-

ing here and there. The character of this lake basin and the type of vegetation which surrounds it are illustrated in fig. 13. On the west of Lake Mary is a steep rugged ridge, which separates it from the glacial basin containing the Twin Lakes. This ridge is made up of smooth glaciated boulders which are very difficult to climb. In order to reach this third tributary cirque the trip must be made



FIG. 12.—Lake Martha with island, Lake Mary in distance, and glaciated mountain side, Big Cottonwood Canyon.

via the main basin, therefore, but no discussion of the main basin will be made until after the tributaries have been discussed.

The Twin Lake basin, like Lake Mary, has been utilized by man for a water reservoir. This basin is the largest of the tributary basins studied, and the lakes are so deep that bottom has never been sounded. The shore vegetation here, as around Lake Mary, has been greatly modified. The only herbaceous flora left near the lake is that which follows the courses of the streams coming from the springs in a small cirque just southeast of the lakes. Along these streams at an altitude of 9500 feet is a hydro-mesophytic vegetation. Here were collected a large number of mosses which will be treated

in a subsequent paper. This region is well covered by willows, a rich undergrowth of grasses, and such plants as *Mimulus Langsdorffii*, *Parnassia fimbriata*, and *Pyrola uliginosa*.

On the northwest side of the lake is a rocky ridge along the top of which *Pinus flexilis* is dominant, as it is on most of the rocky ridges within this region. Fig. 14 shows the characteristic habitat



FIG. 13.—Crevice vegetation around Lake Mary, Big Cottonwood Canyon

and growth of *Symphoricarpus porophilus*, *Lonicera utahensis*, *Dasiophora fruticosa* and the two *Ribes* species so prevalent in this region. The herbaceous plants here are *Aquilegia flavescens*, *Lappula floribunda*, *Castilleja sulphurea*, *C. lancifolia*, *Castilleja* sp., *Lupinus* sp., *Linum Kingii*, *Orthocarpus Tolmiei*, *Phlox caespitosa*, and *Mertensia ciliata*.

The west ridge drops down into a hanging valley which contains the fourth lake studied. This is Lake Solitude, so called because it is the farthest away from the main cirque and the other lakes. This

lake has almost disappeared, as it is being gradually filled by plants and humus. Fig. 7 shows it to be on a substratum of undifferentiated limestone, which has eroded comparatively easily and formed a soil suitable to plant growth. Excepting the main catchment basin, this little cirque holding Lake Solitude is the most densely vegetated of all the glacial cirques studied. The willows, aspens, and conifers

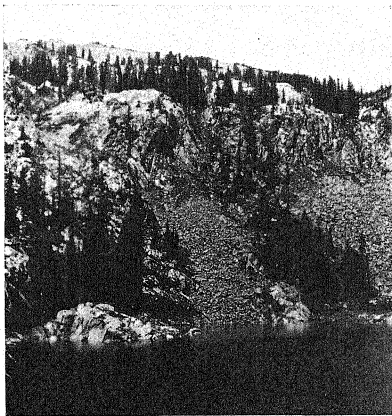


FIG. 14.—View of Twin Lakes, showing rock talus and depressions where vegetation has gained a foothold.

come to the water's edge. On all the surrounding slopes there is a scattering of *Populus tremuloides*, with *Picea Engelmannii*, *Pseudotsuga mucronata*, and *Abies concolor* appearing here and there. On the rocky ridges of the slope, as elsewhere, *Pinus flexilis* still persists. The mountain just east of the lake has on its north-facing slope a very good stand of the conifers typical of the montane forest, with a very mesophytic undergrowth. The spruce-fir forest extends along the slopes from Lake Solitude into the main cirque, where it forms the dominating type of vegetation. Occurring with the other conifers here is *Picea pungens*.

As shown in fig. 7, the main basin has a substratum of glacial alluvium, which is fertile and in undisturbed places is well covered by a xero-mesophytic vegetation. The floor of this basin has an altitude of 8750 feet, which makes the growing season limited to about three months' duration. The main shrubs found here are *Salix Fendleriana*, *S. cordata*, *S. Scouleriana*, *Lonicera involucrata*, *L. utahensis*, *Sambucus microbotrys*, *S. glauca*, *Sorbus scopulina*, *Ribes saxosum*, *R. lentum*, *Rubus strigosus*, *Bossekia parviflora*, *Physocarpus malvaceus*, *Rosa chrysocarpa*, *R. Nutkana*, *R. Fendleri*, and *R. Woodsii*.

The herbaceous undergrowth is a particularly beautiful one because of the luxuriant growth of the individual plants. *Mertensia longistylis*, *Geranium Richardsonii*, *G. Fremontii*, *Potentilla glaucophylla*, *Castilleja lauta*, *Aconitum ramosum*, *Delphinium reticulatum*, *Pedicularis racemosa*, *Aquilegia coerulea*, *Ranunculus maximus*, and *Lappula floribunda* all grow to be three to four feet high, especially in the moister places such as near the streams or around the numerous springs which flow into this basin. As in most mountain resorts, however, this flora is disappearing at a deplorable rate due to the unthinking depredations of the "nature lovers." Overgrazed patches are springing up into groups of *Veratrum speciosum* and *Frasera speciosa*, and *Rudbeckia occidentalis* is becoming much too prevalent.

Where the trees are thickest and the ground is shaded and moist there are many interesting underplants, such as orchids, and species of *Pyrola*, *P. uliginosa*, *P. secunda*, *P. chlorantha*, *Limnorchis*, *Mitella pentandra*, and *Tellima parviflora*. There are only a few kinds of ferns in this region: *Woodsia* and *Pellaea* that have been named as growing in the rock crevices, *Pteridium aquilinum*, and *Filix fragilis*, which is found in only one place in the main canyon. It was formerly found in several places, but tree cutting and changing water courses have caused it nearly to disappear.

The mesophytic vegetation continues down the canyon along the stream course for a distance of about four or five miles, when conditions gradually change. Taking the place of *Pinus flexilis* along the mountain ridges is *Cercocarpus*, and lower on the mountain slopes are to be found *Ceanothus velutinus* and *Quercus Gambellii*. *Pseudotsuga mucronata* is the most persistent conifer, and extends down the

canyon farther than any of the other evergreens. At about 7500 feet altitude the stream vegetation which has been named as characteristic of the other canyons becomes the dominating feature, and continues down into Salt Lake Valley.

Pinus scopulorum is reported as occurring naturally on only one site, which is on the south-facing slope at an altitude of about 6400 feet (8). I did not see this natural stand of yellow pine, but I did see scattered clumps of seedlings planted by the United States Forestry Service, which has a station in Big Cottonwood Canyon at an altitude of 7400 feet. These pines have therefore not been listed in the native vegetation. The isolated groups of native *Pinus scopulorum* make an interesting problem for future investigation. There are also a few yellow pines occurring in Parley's Canyon, which is a few miles north of Big Cottonwood Canyon. It has been reported that these trees of *P. scopulorum* are the farthest north until those of Idaho and Wyoming are reached.

Climatic conditions

PRECIPITATION.—The district covered by this survey is in the semi-arid section of the United States, but the wide variation in altitude results in widely differing amounts of precipitation in different parts of the area studied. At the level of Salt Lake City, 4408 feet, the average precipitation, most of which is rainfall, is about 16 inches, while at the head of Big Cottonwood Canyon at 8700 feet it is about 43 inches, of which a good deal is snowfall. ALTER'S (2) study of the relation between precipitation and altitude shows that on the western slopes of the Wasatch Mountains there is an increase of about 4 inches per year per 1000 feet.

TEMPERATURE.—Very wide variations of temperature are found in the area herein considered. These variations are not only seasonal but diurnal. Even at the level of Salt Lake City the annual temperatures vary from a summer maximum of about 107° F. to winter temperatures of about zero. Very much lower winter temperatures are of course found at the higher altitudes, and diurnal variations are of course greater there also.

The annual mean temperature taken from 45 years' record at Salt Lake City is 51.6° F., while the mean average from May to

October as recorded at the Big Cottonwood Nursery (7450 feet in altitude) is 51.9° F. The mean average for the same months in Salt Lake City is 65.2° F. Air temperatures decrease quite uniformly with increase in altitude, and it is thought that the usual relation holds in a general way here; that is, that the temperature gradient is about 3.5° F. for each 1000 feet (2). Table I gives the mean temperatures by months in degrees F. for Salt Lake City and Big Cottonwood Nursery, Utah (2).

TABLE I
MEAN TEMPERATURES

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
Salt Lake City, altitude 4408.....	29.2	33.4	41.8	49.9	57.4	67.1	75.6	74.6	64.4	52.5	40.9	32.1
Big Cottonwood Nursery, altitude 7450....	42.5	55.2	62.4	60.0	51.8	39.5

From the preceding data it can be seen that in Salt Lake City there are usually four months of the year (November, December, January, and February) that are below 40° F. and hence unsuitable to plant growth; while at an altitude of 7450 feet there are six months with temperatures too low to permit vegetation. At the highest altitude reached in this investigation the growing season probably does not exceed three months.

EVAPORATION.—Evaporation is very rapid because of the high summer temperatures and low humidity. The average annual evaporation on the shores of Great Salt Lake is about 80 inches, consequently in this region we get plants of desert types. In the canyons evaporation is lessened on account of the decrease in temperature as compared with the evaporation in the valley. Also, greater summer precipitation in the canyons tends to offset evaporative action, so that at an elevation of 7500 feet the net evaporation from June to September averages about 24 inches. In the canyon bottoms and on the lower slopes, the effects of evaporation are offset by the water supply from the springs and streams, a condition which is not attained either at a higher elevation or in the valley. The effects of evaporation, therefore, are among the more important factors affecting the type of summer flora found in the different parts of the region studied.

Summary

1. **SALT LAKE VALLEY** (extreme eastern portion of Great Basin).—This is occupied by two different types of vegetational composition, due to soil conditions. The soil on the west side of the Jordan River being of alkaline nature, is covered by a sparse growth of alkali-tolerant plants. The soil on the east of the Jordan River is higher, well drained and fertile, and is mostly cultivated.

2. **UNGLACIATED CANYONS**.—These show a succession from the grass covered foothills, through sage brush and oak chaparral to an aspen-spruce forest at their heads. The City Creek Canyon which has a west-by-south direction is xerophytic for a longer distance up the canyon on the south-facing slopes. Emigration Canyon, which has a southwest direction, shows practically the same kind of vegetation on both slopes of the canyon; the east-facing slope may be a little more mesophytic than the west, since the aspens come in somewhat lower on the east face.

3. **GLACIATED CANYONS**.—Where not disturbed by fire, overgrazing, and overcutting, these show the same vegetational successions as the unglaciated canyons. These canyons are more rugged, however, are deeper, and go to higher altitudes, developing on the higher north-facing slopes a good stand of the spruce-fir montane forest, which gives way at about 10,000 feet to a subalpine flora. The area here reaches just a few feet above timber line.

Conclusions

The differences that occur in the vegetational cover of Salt Lake Valley and the lower adjacent canyons are due almost entirely to edaphic factors. In the valley the differentiation is caused mainly by the accumulation of alkali salts in the land west of the Jordan River. On the fertile or salt-free land east of the river the limiting factor seems to be water, for where water is supplied the canyon vegetation extends into the valley. Also the fact that with irrigation such mesophytic crops as peaches and apricots grow successfully would indicate that water might be the limiting factor.

The changes which occur in the character of the vegetation as one ascends the canyons in all probability are due to the gradual changes in climate and soil conditions. The high ridges are mostly rock and intercept the strong westerly winds of this region, so that

one would expect only the wind forms and the xerophytic type of vegetation which are found there. The observations of other ecologists were confirmed to the effect that the north-facing slopes retain more moisture than do the south-facing slopes, and hence show a greater development of the mesophytic type of vegetation.

The native vegetation of the entire region has been greatly modified by the activities of civilization: for instance, great areas have been subjected to over-grazing by cattle and sheep; the scant timber supply has had to be used for man's immediate need of housing and fuel; much of the land has been put under cultivation, and many of the streams and lakes have been utilized for domestic and industrial purpose. It appears logical, therefore, to conclude that many of the factors influencing the character of the vegetation described herein are those of a biotic nature.

Notwithstanding the modifying effects of agriculture, logging operations, and grazing, however, there remains ample evidence that the native flora in the area studied is determined by the natural conditions of soil, altitude, precipitation, temperature, evaporation, and length of growing season, and varies in type and characteristics as these factors vary. This is shown by the changes in the native flora from desert forms to subalpine types, depending on location as described and listed in this study.

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SEASONAL DEVELOPMENT OF GROWTH LAYERS IN *FRAXINUS CAMPESTRIS* AND *ACER SACCHARINUM*

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(WITH PLATES XIX-XXI AND TWO FIGURES)

Introduction

Considerable investigation has been done on the increase in diameter of trees. The literature up to 1915 has been excellently summarized by GROSSENBACHER (7). Much work remains to be done, however, as we know little or nothing about the behavior of many species, about the behavior of trees in many regions, and the behavior of the same species in different seasons. The aims of this study were to discover when xylem formation began and ended, the rate at which new xylem cells were produced, and to compare the course of xylem formation with environmental factors, to see whether there was any correlation. The trees used were *Fraxinus campestris* Britton, forming ring-porous wood, and *Acer saccharinum* L., forming diffuse-porous wood. This study was carried on during the growing season of 1925, in the vicinity of Lincoln, Nebraska.

Methods

Trees were cut down at intervals from March 23 to October 14. The ash trees were growing in an open forest on the floodplain of Salt Creek, about 3 miles southwest of Lincoln. The soft maples grew in a grove of small trees and shrubs in rich soil on a vacant lot on the upland, about 5 miles northeast of Lincoln. The ash trees ranged from 10 to 40 years in age, the soft maples from 8 to 14 years. Small blocks about an inch long, half an inch wide, and a quarter of an inch thick, containing the outer xylem and the inner phloem, were cut from a height of one foot above the soil. Blocks were taken in duplicate from opposite sides of the trunk on each

¹Miss BERNICE BRENKE prepared the slides and helped considerably with the drawings; the senior author did the rest of the work and assumes responsibility for all statements.

date, and were kept in a solution containing equal parts of glycerine and 95 per cent alcohol before cutting sections on a sliding microtome. The sections were cut 30-40 μ thick, immersed for a short time in Eau de Javelle to remove the protoplasmic contents of the cells, stained with safranin and Delafield's haematoxylin, and then permanently mounted. This procedure made the cell walls stand out clearly, and showed good differentiation between the lignified and cellulose walls.

Although a different tree was used on each date, the results of the study appear important enough to warrant publication, especially since very little or no work of this sort has been done on these two species, so far as the writers could determine. Investigators have shown that trees of the same species, even when growing close together, vary considerably in xylem production. The advantage in using a different tree on each date, therefore, is that the general course of development of a number of individuals is secured in place of the development of a single tree which might be abnormal in its behavior. Since fourteen determinations were made for each species during the growing season, the sections were taken closely enough together so that they serve as checks upon one another.

Development in *Fraxinus campestris*

MARCH 28.—The buds were only slightly swollen, no green showing (fig. 1). The bark adhered firmly to the wood. The width of the cambium was 4-6 cells, averaging about 31.5 μ . No new xylem cells had formed. The age of the tree was 33 years; diameter 4.5 inches.

APRIL 15.—The tree was in full bloom, the leaves were just beginning to expand but showed no green (fig. 2). The bark separated from the wood at the cambium very easily, leaving the exposed surface rather watery. Age 35 years; diameter 5 inches.

The width of the cambium layer was 4-6 cells, about 35 μ . The maximum width of the new xylem was 88 μ ; the largest tracheary tubes were 105 μ in tangential diameter. Tracheary tubes were forming rapidly.

APRIL 20.—The leaves on the tree were about 1.2 inches long, the longest terminal growth of twigs 0.5 inch (fig. 3). The bark did not separate from the wood quite as easily as on April 15, but the

newly exposed surface was very wet and somewhat viscous. Age about 40 years; diameter 4.5 inches.

The thickness of the cambium was 5-8 cells, about $45.5\ \mu$. The maximum width of the xylem was $189\ \mu$; the maximum diameter of the tracheary tubes was $189 \times 235\ \mu$. The tracheary tubes were developing rapidly, and the walls of the larger tracheary tubes were a little thicker than those of the other cells.

APRIL 27.—The oldest pair of leaves were 5-7 inches long (including the petiole); the largest leaflets (lowest pair on a leaf) were 1×3 inches, the upper pairs were smaller, $0.5-0.7 \times 1.5-2.0$ inches. The tree appeared fairly leafy (fig. 4). Terminal growth of twigs averaged about 3.5 inches long. Age of tree about 45 years; diameter 5.2 inches.

The thickness of the cambium was 5-9 cells, about $52.5\ \mu$. The maximum width of the xylem was $360\ \mu$. The innermost row of tracheary tubes had reached full size ($225 \times 240\ \mu$), and the walls of most of them were thickened ($3.5-7\ \mu$ thick) and lignified like the mature tubes of the preceding year. The other large tubes, nearer the cambium, were enlarging rapidly. Often the first row and in places some of the cells in the second row of cells surrounding the lignified tracheary tubes were beginning to lignify their walls. The development of the new xylem in one-year old and 1925 twigs was about the same as at the base of the trunk, except that lignification was slightly more advanced and the ducts were much smaller and more numerous in the twigs.

MAY 4.—The lowest three pairs of leaves on the twigs were usually 5-7 inches long, with leaflets averaging about 1×3 inches in area. The increase since April 27 in leaf area had been great. Individuals varied considerably in the rate of leafing out. The fruits on this tree were about one-eighth of an inch long. The terminal growth of the twigs varied from 1 to 5 inches, and on some twigs terminal and axillary buds had formed, but on other twigs leaves were still being produced. This tree was 5.5 inches in diameter and 45 years old.

The width of the cambium was 5-9 cells, averaging about $52.5\ \mu$. The maximum width of the xylem was $345\ \mu$, and the inner part, about $150-225\ \mu$ wide, was partially thickened and lignified. The

first row or two of cells surrounding the inner tracheary tubes had walls about $3.5\ \mu$ thick, and the rest of the cells between the tracheary tubes had walls about $1.8\ \mu$ thick. The thickness of the walls of the tracheary tubes was similar to those of the April 27 specimen. The outer edge of the lignified region was wavy, due to the earlier thickening and lignification of the tracheary tubes followed by the

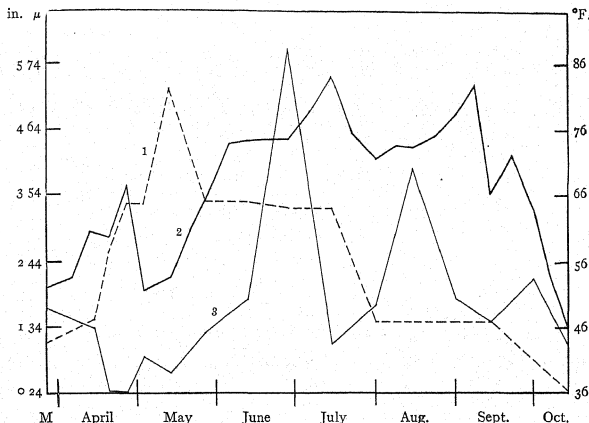


FIG. 1.—1, Width of cambium layer in *Fraxinus campestris* in microns during season of 1925; 2, mean temperature for intervals of about 7 days; 3, total precipitation for intervals between cambium measurements.

wood fibers between them. All of the large tracheary tubes (reaching a diameter of $225\ \mu$) were fully developed, and only the outer ones ($90\ \mu$ or more) outside of the 1924 xylem were unlignified. Many smaller tubes, $75\ \mu$ or less in diameter, were being formed outside of the large ones, and these showed no lignification.

MAY 14.—The lowest two pairs of leaves on the twigs were usually 6–7 inches long, the rest 3–5 inches (fig. 5). The leaflets on the lower two pairs of leaves were about 1×3 inches in area. The

tree appeared quite leafy, but some of the leaves were not fully grown yet, and different individuals still showed considerable variation in leaf development. The terminal growth of the twigs varied from 1 to 4.5 inches. The diameter of the tree was about 5 inches; age 14 years.

The width of the cambium was 7-9 cells, about $70\ \mu$. The maximum width of the xylem was $750\ \mu$, the inner lignified part being $450-750\ \mu$ wide. All of the large ducts (2 or 3 rows) were fully thickened and lignified, reaching radial diameters of $300\ \mu$. The cells immediately adjacent to these ducts had walls about $3.5\ \mu$ thick, but the other cells between the ducts in the lignified part had walls $1.8\ \mu$ or less thick. The activity of the cambium was great at this time.

MAY 27.—The lowest three or four pairs of leaves on the twigs were 6-8 inches long (including the petiole). The leaflets averaged about 1.2×3 inches in area. Many young leaves and stem tips were brown or black from the effects of frost. The terminal growth of the twigs ranged up to 14 inches. The tree appeared quite leafy. The diameter was about 4.5 inches; age 14 years.

The width of the cambium was 7-9 cells, about $53\ \mu$. The maximum width of the xylem was $645\ \mu$, the inner part ($330-570\ \mu$ wide) partially lignified. There were 1.5-2 rows of large ducts, the largest $225\ \mu$ in diameter, all fully thickened and lignified except an occasional one farther out. The cells between the tracheary tubes, except for the row or two surrounding the tubes, were usually about $1.8\ \mu$ thick. Many small tracheary tubes were being formed, and the activity of the cambium was still great.

JUNE 13.—The leaves appeared fully grown, the lower pairs averaging about 8 inches long with leaflets about 1.2×4 inches (fig. 6). The terminal pairs were smaller and no new leaves were being formed. The tree was very leafy. The terminal growth of the twigs was 2-8 inches. The twigs were stiffer than on May 27, brown streaks and blotches were developing in the bark, and the lenticels were white and prominent. The diameter was 5.2 inches; age 29 years.

The thickness of the cambium was 7-9 cells, about $53\ \mu$. The maximum width of the 1925 xylem was $720\ \mu$, the partially lignified

part 450–645 μ . There were 2 or 3 rows of large ducts, the largest 225 μ in diameter, all of which had fully thickened and lignified walls. Many of the smaller ducts (about 60 μ in diameter) were also fully thickened and lignified (walls 5–7 μ thick), and partially lignified ducts were found only 2–4 cells away from the cambium. The walls of the cells between the large ducts averaged about 3.5 μ thick, and these thick walled cells extended out, forming a row or two around many of the small ducts. The walls of the other cells between the ducts were about 1.8 μ thick. The activity of the cambium appears to have become retarded, but new cells, including a number of small ducts, were still being formed. The zone of unthickened and unlignified cells was much narrower than before, so that lignification had been going on rapidly.

JUNE 28.—Due perhaps to the rainy weather in the preceding two weeks, renewed growth from terminal buds had produced clusters of up to 5 or 6 small, tender leaves on new stems (fig. 7). The new terminal growth was 1–2 inches long. The dry weather before June 13 may have caused premature development of the terminal buds. The leaves below these clusters of new leaves were fully developed, as indicated by their stiffness, color, and size. If the dry weather had continued it is likely that no new leaves or terminal growth would have been produced. The 1925 portions of the twigs were fairly stiff, but could be bent beyond 90° without breaking. The brown spots in the bark of the 1925 twigs were larger than on June 13, the yellow or brownish tinge of the entire twigs was more pronounced, and the conspicuous lenticels were pale brown. The diameter of the trunk was 4.5 inches; age 20 years.

The cambium was 7–9 cells wide, about 52 μ . The maximum width of the xylem was 1710 μ . The lignified part of the xylem was 1560 μ wide, 70 per cent (forming an inner zone) being fully lignified, with walls 3.5–7 μ thick. The rest of the lignified cells between the ducts had walls about 1.8 μ thick. There were 2 or 3 rows of large ducts, and the small ducts were fully lignified to within 150 μ of the cambium. The activity of the cambium had lessened considerably, but new ducts and other cells were still being formed. Lignification had been progressing very rapidly, the unthickened and unlignified zone averaging only about 150 μ in width.

JULY 15.—Leaves were fully developed and no new ones were forming (fig. 8). The terminal buds for 1926 were about 0.5 inch long. The terminal growth of the twigs was as much as 11-15 inches, and elongation had ceased. The bark was greenish brown, slightly greener than the 1924 portions. The lower 3-6 inches of the 1925 growth broke with a snap rather easily instead of bending, but the upper part could be bent beyond 90° without breaking. The lenticels formed conspicuous whitish streaks up to about 0.25 inch long. The sap at the cambium was more viscous and less watery than heretofore. The diameter was 5.7 inches; age 33 years.

The cambium on the sides of the trunk which were examined was about 8 cells wide, or about $52\ \mu$. The maximum width of the xylem on one side of the trunk was $1875\ \mu$, of which about $1725\ \mu$ was lignified. An inner zone, 1125 - $1425\ \mu$ wide, was completely lignified (walls 4 - $7\ \mu$ thick), and the outer zone of the lignified portion, 300 - $600\ \mu$ wide, was considerably lignified (walls 3.5 - $5\ \mu$ thick). A narrow zone, only 75 - $150\ \mu$ wide, was unlignified. Cambial activity had lessened so that only a few new cells were being formed. On the opposite side of the trunk the cambium had apparently ceased producing new cells, and the xylem, maximum width $975\ \mu$, was lignified to the cambium. The cells in the outer zone of the xylem, 125 - $140\ \mu$ wide, had walls 1.8 - $3\ \mu$ thick; in the next inner zone (about $115\ \mu$ wide) the walls were about $3.5\ \mu$ thick; and in the inner zone they were 4 - $7\ \mu$ thick. Lignification was being completed rapidly.

AUGUST 1.—No new leaves had appeared (figs. 9, 10). The terminal buds were well developed. The 1925 part of the twigs was all brownish gray, but was more pliable than the 1924 part, as most of it could be bent beyond 90° without breaking. The sap at the cambium was less watery than on July 15. The diameter was 4 inches; age 19 years.

The cambium was 6 or 7 cells wide, about $35\ \mu$. On one side of the trunk the xylem, reaching a maximum width of $675\ \mu$, was completely thickened and lignified to the cambium. Although the outermost xylem cells were narrow like the last ones formed during the season, an occasional new cell was developing. On the opposite side

of the trunk the formation of xylem cells had ceased and had later started anew. This was shown by the layer (about $56\ \mu$ wide) of narrow cells with thick walls inside of the cambium. This outer layer of xylem consisted of larger cells with very thin walls. The remainder of the xylem was completely lignified (walls $4\text{--}7\ \mu$ thick) except the outer portion, about $70\ \mu$ wide, which had walls that were usually $3.5\ \mu$ wide. The maximum width of the xylem on this side was $960\ \mu$, 88 per cent of which was fully lignified.

AUGUST 15.—No new leaves had appeared (fig. 11). The terminal growth this year showed as much as 10–16 inches' elongation. The distal 4–8 inches had come from the renewed activity of the terminal bud. The 1925 part of the twigs broke with a snap when bent as far as 90° . The sap at the cambium was quite viscous. The diameter was 4.2 inches; age 10 years.

The cambium was 6 or 7 cells wide, about $35\ \mu$. The maximum width of the xylem on one side was $3825\ \mu$ and the walls were completely thickened and lignified ($3.5\text{--}7\ \mu$ thick). Occasionally a narrow cell adjacent to the cambium appeared to be still thickening its walls. On the opposite side of the trunk, where the maximum width of the xylem was over $5250\ \mu$, the cambium was slightly more active because there was a zone of xylem, about $35\ \mu$ wide, adjacent to it with walls only slightly lignified. The rest of the xylem was completely lignified. On the whole, the formation of new xylem cells had almost ceased in this tree.

SEPTEMBER 14.—The leaves were quite hard and stiff, a few had turned brown, and brown spots were common (fig. 12). The upper 2–4 leaves on the twigs had fallen, and the terminal 0.5–1 inch of the stems was dry. The 1925 part of the twigs resembled the 1924 part in color and brittleness. The buds were deep brown. The sap at the cambium was much drier than heretofore. The diameter was 5 inches; age 11 years.

The cambium was 6 or 7 cells wide, about $35\ \mu$. The new xylem on one side was $5250\ \mu$ wide; on the opposite side $4500\ \mu$. It was completely lignified, and the demarcation of xylem and cambium was very sharp. Formation of new xylem cells had ceased.

OCTOBER 14.—The leaves had fallen. The bark did not separate as easily at the cambium as before, and there was considerable moisture in the outer xylem and the inner phloem. The diameter was 5.5 inches; age 36 years.

The cambium was 3–5 cells wide, about $24\ \mu$. The xylem in this tree was only $1925\ \mu$ wide, all of which was completely lignified.

Development in *Acer saccharinum*

MARCH 23.—The tree was in full bloom but the leaves were not yet out. The trunk was very full of sap, the sawdust forming a pasty mixture with it. The diameter was 4.5 inches; age 10 years.

The cambium was fairly thick walled, 3 or 4 cells wide, or about $17\ \mu$. No new xylem.

APRIL 13.—The leaves averaged about 1×1 inch, pale green or reddish in color (fig. 13). The sap was not so watery as on March 23. There was considerable variation in the rate of leafing out, some trees having no leaves yet out and some with leaves 2×2 inches in area. The diameter was 4 inches; age 8 years.

Cambium was the same as on March 23; no new xylem.

APRIL 20.—The leaves averaged about 2×2 inches, were very thin, and somewhat reddish or yellowish green (figs. 14, 15). The stems had elongated up to 0.5 inch. The bark separated from the wood at the cambium readily, leaving the exposed surface very watery and slightly viscous. Sap was not so watery as on March 23. The fruit was about full grown but green. The diameter of the tree was 3.7 inches; age 7 years.

On one side of the trunk the cambium was about 12 cells wide, or $52\ \mu$. The maximum width of the new xylem was about $105\ \mu$, or about 10–12 cells. The largest tracheary tubes were $32\ \mu$ in diameter. All the cell walls were very thin. Growth on this side had been going on for only a short time, and was now proceeding very rapidly. On the opposite side of the trunk the cambium was about 8 cells wide, $35\ \mu$. Production of new xylem cells had just started, one row about $10\ \mu$ wide having been formed. No tracheary tubes could be distinguished.

APRIL 27.—The leaves averaged about 2.5×3 inches and had a good green color. The greatest elongation of the twigs was about 2

inches. The fruits were full grown and beginning to dry. The sap was very watery. The diameter was 4.5 inches; age 12 years.

The cambium was about 8 cells wide, or $32\ \mu$. Growth had just started. Only occasionally had a new xylem cell reached full size.

At a height of 8 feet from the ground, where the trunk was 3 inches in diameter and 9 years old, the new xylem was about $52\ \mu$ wide. There was a conspicuous ring of tracheary tubes with tangential diameters up to $52\ \mu$. The walls of all the cells were very thin, and lignification had not started. The cambium was 12–15 cells wide, about $70\ \mu$.

At a height of 18 feet from the ground, where the main stem was 2.5 inches in diameter and 4 years old, the new xylem was 80–105 μ wide (fig. 16). There were many tracheary tubes up to $52\ \mu$ in diameter, the inner ones having slightly thicker walls than the rest of the xylem. The cambium was 12–15 cells wide, about $70\ \mu$.

In one-year old twigs the new xylem was 70–105 μ wide, with many ducts up to $52\ \mu$ in diameter (fig. 17). The walls of most of the ducts in the inner 48 μ of the new xylem were partly thickened and lignified, about $1.7\ \mu$ thick. The cells immediately surrounding these tubes had slightly thickened walls. The cambium was about 10 cells wide, 35–70 μ .

The cross-section of the 1925 portions of the twigs resembled the cross-section of an herbaceous stem, in that the bundles were separated (fig. 18). The xylem in some of the bundles reached 175 μ in width, of which about 140 μ were partly thickened and lignified. The walls of the tracheary tubes averaged about $3.5\ \mu$ in thickness. The maximum diameter of the tracheary tubes was about $32\ \mu$. The cambium was 7–9 cells wide, 35–52 μ .

MAY 7.—The lowest two pairs of leaves on the twigs were about 3.5×4.5 inches, the upper ones were smaller (fig. 19). The tree appeared quite leafy. There was much sap in the wood. The terminal growth varied from 0.7 to 2.5 inches in length. New buds were just beginning to appear.

The cambium on one side of the trunk was about 20–25 cells wide, about 135 μ . The maximum width of the new xylem was 390 μ , of which the walls of the inner part, 135 μ wide, were slightly thickened and lignified (walls about $1\ \mu$ thick). The tracheary tubes

in this zone had reached full size, the largest being about $75\ \mu$ in diameter. On the opposite side of the trunk the cambium was about 15 cells, or $75\ \mu$ thick. Cell division was very rapid at this time.

MAY 14.—Many of the leaves appeared mature, as they were 5 to 6 inches broad by about 4 inches long; they were much stiffer than before. New leaves were being produced. The elongation of

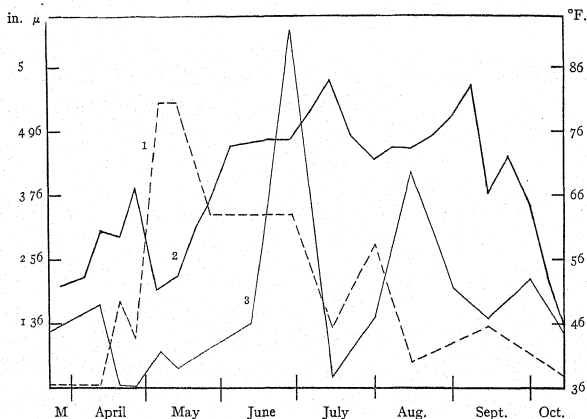


FIG. 2.—1, Width of cambium layer in *Acer saccharinum* in microns during season of 1925; 2, mean temperature; 3, total precipitation.

the terminals ranged from 1 to 3.5 inches. The diameter was about 4 inches; age 14 years.

The cambium was about 15 cells wide, or $105\ \mu$. The new xylem was $420\ \mu$ wide. The inner part, about $300\ \mu$ wide, was partly thickened and slightly lignified. The inner half of the lignified part had walls $1.7\text{--}3.5\ \mu$ thick. The walls of the ducts were similar to those of the other xylem cells. The formation of new xylem cells was proceeding very rapidly.

MAY 27.—Most of the leaves, about $4 \times 4\text{--}6$ inches in area, were mature, as indicated by the deep green color, thickness, and firmness

of the blades (fig. 20). New leaves were being formed. Elongation of the terminals was up to 8.5 inches. The diameter of the tree was 3.5 inches; age 10 years.

The cambium was 12–15 cells wide, about $70\ \mu$. The width of the new xylem was $990\ \mu$. The inner part, about $825\ \mu$ wide, was partly thickened and lignified. Two zones could be distinguished in this, the inner about $522\ \mu$ wide with walls about $3.5\ \mu$ thick, and the outer about $303\ \mu$ wide with walls $1\text{--}1.5\ \mu$ thick. Cell division, thickening, and lignification were proceeding rapidly.

JUNE 13.—New leaves were still appearing, but the rate of the formation of new leaves had decreased considerably (fig. 21). The tree was very leafy. The terminal twigs had elongated up to 14 inches. The lower half of the new terminal growth had conspicuous brown lenticels, the upper half had white lenticels. The portion of the twigs with brown lenticels was much stiffer than the other part. The diameter was 4.5 inches; age 10 years.

The cambium was about 15 cells wide, or $70\ \mu$. The xylem was $2775\ \mu$ wide, of which only a narrow zone, $180\ \mu$ wide, adjacent to the cambium was thin walled and unligified. The innermost zone of the xylem, about $2222\ \mu$ wide, had walls $3.5\ \mu$ thick but was not mature; the outer zone, about $375\ \mu$ wide, had walls $1.2\text{--}1.7\ \mu$ thick. On the opposite side of the trunk the xylem was $2730\ \mu$ wide. Cell division was very active on this date, and lignification was proceeding rapidly.

JUNE 29.—More leaves had reached maturity (fig. 22). New leaves were still forming and some of the new terminal buds had opened, due perhaps to the moist weather of the preceding two weeks following the previous hot dry period. The twigs were stiffer than on June 13. The diameter was 4 inches; age 8 years.

The cambium was about 15 cells wide, or $70\ \mu$. The 1925 xylem was $2640\ \mu$ wide. The thin walled and unligified zone was about $225\ \mu$ wide. The innermost part of the xylem, about $1590\ \mu$ wide, was fully lignified, with walls $5\text{--}7\ \mu$ thick. The demarcation of this inner zone was very conspicuous. The next outer zone, about $525\ \mu$ wide, had walls $1.8\text{--}3.5\ \mu$ thick. In the outer zone of the lignified part, about $300\ \mu$ wide, the walls were only slightly thickened and lignified. In the opposite side of the trunk the xylem was $2925\ \mu$

wide, of which the inner zone, 1700 μ wide, was fully lignified. Cell division was very active, thickening and lignification of the walls was following rapidly. About 60 per cent of the xylem formed by this date was fully lignified.

JULY 15.—New leaves were still appearing, clusters of young leaves being conspicuous at the ends of many branches (fig. 23). The elongation of the topmost terminals was up to 14 inches. Most of the upper side of the 1925 portions of the twigs had turned brown but the under side was green. Twigs were stiffer than before, but did not break readily when bent. The sap appeared more viscous than before. The diameter was 3.5 inches; age 8 years.

The cambium was 7 or 8 cells wide, about 35 μ . The 1925 xylem was 2445 μ wide. The unlignified and unthickened zone was usually not more than 16 μ wide. The innermost, mature zone of the xylem, walls 5–7 μ thick, was about 1060 μ wide. The zone outside of this one, walls 3.5–5 μ thick, was about 1155 μ wide, and the next zone, about 210 μ wide, showed tracheary tubes with walls 3.5 μ thick and the cells between with walls only slightly thickened and lignified. In the opposite side of the trunk the new xylem was 2625 μ wide, the innermost 930 μ being mature. The production of new cells had decreased considerably, and thickening and lignification were proceeding rapidly.

AUGUST 1.—The number of leaves produced on single twigs ranged up to 73, and many new leaves were still appearing (fig. 24). The terminal growth was up to 24 inches, the distal 3–6 inches having grown from the terminal bud formed this summer. There were many short shoots on the basal part of the 1925 portion of the twigs, having grown from lateral buds formed this summer. The bark was attached more firmly to the wood than before, and the sap at the cambium was much less watery. The diameter was 4.2 inches; age 8 years.

The cambium was 10–14 cells wide, about 61 μ . The new xylem was 6225 μ wide, forming three zones. The innermost zone, about 5790 μ wide, was mature with walls 3.5–7 μ thick; the next one, about 225 μ wide, had walls ranging from only slightly thickened and lignified to 3.5 μ thick, and the outermost zone, about 210 μ wide, had walls only slightly thicker than the cambium. In the opposite

side of the trunk the xylem was about $4665\ \mu$ wide, $4290\ \mu$ being mature. Cell division was still very active in this tree, and complete lignification was far advanced, about 92 per cent of the xylem being mature.

AUGUST 15.—New leaves had ceased appearing (fig. 25). The smallest leaves were about 1×1 inch in area, but all appeared mature or nearly so, as indicated by the degree of cutinization, firmness, and white undersurface of the blades. The buds for 1926 were quite prominent, about 2.5 mm. long at the tips of the branches. The elongation of the twigs was up to 15 inches. The 1925 part of the twigs could still be bent beyond 90° without breaking. The sap at the cambium was viscous. The diameter was 5 inches; age 12 years.

The cambium was about 5 cells wide, or $24\ \mu$. The 1925 xylem was $4440\ \mu$ wide and completely lignified to the cambium. The walls of the cells bordering the cambium were $3.5\text{--}5.2\ \mu$ thick, and the cells were narrow like the last formed during the season. The other side of the trunk was similar. Cell division had ceased.

SEPTEMBER 14.—The leaves were hard, stiff, and brittle, and no new ones had appeared since August 15 (fig. 26). The 1925 portions of the twigs were deep brown. They were brittle, breaking readily when bent beyond 90° , except the distal 1-3 inches, which was still green and very pliable. The sap at the cambium was much less abundant. The diameter was 3.5 inches; age 8 years.

The cambium was about 7 cells or $35\ \mu$. The 1925 xylem was $2580\ \mu$ thick, completely lignified except for an outer zone about $60\ \mu$ wide, which was thin walled and unlignified. In this thin walled zone new tracheary tubes were being formed as well as other xylem cells. On the opposite side of the trunk the xylem was $2015\ \mu$ wide, and the thin walled zone about $30\ \mu$ wide. In this tree cell division had ceased for the season, as indicated by the narrow, thick walled cells on the outer edge of the mature xylem, and then it had resumed again to produce the thin walled zone of larger cells bordering the cambium.

OCTOBER 15.—The leaves were still green on the tree examined, but on many trees the leaves were turning yellow. The bark did not separate as readily as earlier, but the cambium region still contained much water. The diameter was 3.2 inches; age 8 years.

The cambium was 4 or 5 cells wide, about $19\ \mu$. The xylem was $1920\ \mu$ wide, on the opposite side $1395\ \mu$, completely thickened and lignified. Cell division had ceased.

Relation of cell formation to environmental factors

The precipitation and the temperatures for the season were secured from the United States Weather Bureau. The precipitation record for the maple trees is the average of two stations, one two miles southwest of the grove, the other two miles northeast. The temperature record was secured about four miles southwest. For the ash both records were secured from the station about three miles northeast of the grove. The mean temperature and the total precipitation for periods corresponding to the intervals at which sections were taken from the trees are shown graphically in figs. 1 and 2. The width of the cambium layer on fourteen different dates was used for the growth record. Although the section for each date was taken from a different individual, the curve representing the variation in the width of this layer is an index to the rate at which new xylem cells were being formed. The wider the layer the more rapidly were new cells forming, and vice versa. The width of the xylem could not be used as a growth record because this varied too much, even in individuals growing side by side.

In order to determine the variation of the cambium layer at a certain time, the main stems of five young trees of each species were examined on December 1. In ash trees ranging from two to four years old, the width of the cambium was 3-6 cells, or $17-24\ \mu$. The width of the 1925 xylem varied from 225 to $780\ \mu$. In the maple trees 3-5 years old, the width of the cambium was 3-5 cells, or about $14-18\ \mu$. The width of the 1925 xylem was $300-645\ \mu$, so that the variation in these trees was slight.

No relation is apparent in either species between the precipitation curve and the width of the cambium. In the period preceding June 27-28, and again on August 15 when the rainfall was high (over 5 and 3 inches respectively), the rate of the production of new xylem cells remained stationary or decreased. A correlation seemed to exist between cambial activity and mean temperature. As the temperature rose above about 52°F. , about April 13, cambial activity began

in the ash, and in the maple activity did not begin until about April 20, when the mean temperature had risen to 60° F. Cambial activity increased very rapidly in both species until the period May 14-27, when the mean temperature reached 65° F. As the temperature rose cambial activity declined gradually and then rapidly. In the period July 15 to August 1 the mean temperature declined from 84° to 75° and 72° F., and the width of the cambium in the maple increased from 35 to 61 μ . As the temperature went up again the cambial activity became slower, and on September 14 cell division was again proceeding accompanied by a fall in temperature.

Lignification was proceeding especially rapidly in both species after June 13, when the mean temperature was above 70° F. The June 28 ash showed that 70 per cent of the xylem formed by that time was completely lignified; in the maple about 60 per cent. Lignification in these two species appeared to be accelerated by higher mean temperatures, at least above 70° F., and cell division appeared to be favored by mean temperatures below 60° F., and retarded by higher ones.

Discussion

The maple was found to be similar to most trees (7, 8, 14) in that it did not show xylem formation until the leaves were partly expanded. The ash, however, formed new xylem before the leaves had started to expand or the branches to elongate. MACDOUGAL (14) states that *Fraxinus arizonica* and *Parkinsonia aculeata* are the only trees that he had encountered which showed trunk enlargement prior to shoot formation.

Early in the season there appeared to be a direct correlation between the mean temperature and cambial activity, and later the correlation appeared to be an inverse one. More study is needed before this can be established. No relation appeared evident between precipitation and cambial activity. It was not possible during the season to study the effects of other factors, such as soil temperature, soil moisture, humidity, wind, etc., but it is hoped that this will be possible in future work. There appears to be much conflicting literature on the relation of diameter increase in trees and environmental factors, but the determining factors would vary in different regions. As MACDOUGAL and SHREVE (13, 14) have emphasized,

the "entire constellation of environmental factors" should be studied in relation to growth. Several individuals in each species studied should be followed throughout the season and the dendro-graphic method should be combined with the histological.

Summary

1. The aim of this study was to follow the course of the seasonal development of the xylem in *Fraxinus campestris* Britton and *Acer saccharinum* L. at Lincoln, Nebraska, by the histological method.

2. In the trunk of the ash, one foot high, xylem formation had started just prior to April 15, when the trees were in full bloom, and before any leaf enlargement had occurred. By April 27 the innermost row of tracheary tubes had reached maturity; by May 4 all of the large tubes were fully grown; and by May 14 all the spring wood had been formed and lignification was proceeding rapidly. The formation of new cells had decreased somewhat on June 13, and considerably so on June 28. By July 15 the summer growth had almost ceased, and over 90 per cent of the xylem was completely lignified. Renewed cambial activity was producing a few cells on August 15 on one side of the trunk, but on the other side it had not started again. On September 14 the formation of new xylem cells had ceased and lignification was complete to the cambium.

3. In the trunk of the maple, one foot high, xylem formation started in one tree just prior to April 20, and in another tree about April 27, when the leaves were about half grown. The production of new cells was very active throughout May and June, but on July 15 it had decreased considerably. By August 1 most of the season's xylem had been formed, and over 90 per cent was completely lignified. On August 15 lignification was complete to the cambium and no new cells were being produced, but on September 14 cambial activity had been renewed and new xylem cells had developed, after growth had apparently ceased for the season. By October 14 cell formation had ceased and the xylem was completely lignified.

4. On April 27 the xylem development in the ash was similar in the base of the trunk and in the 1924 and 1925 portions of the twigs, except that lignification was slightly more advanced, and the tracheary tubes were smaller and more numerous in the twigs. In the

maple on April 27 xylem formation was much further advanced in the young twigs than in the trunk, and it decreased progressively as the base was approached.

5. Cambial activity, as measured by the width of the cambium layer composed of the narrow, thin walled cells, showed no correlation with precipitation. With mean temperature, however, a direct correlation appeared to exist in the spring, until about 60° F. was reached, after which there appeared to be an inverse correlation.

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EXPLANATION OF PLATES XIX-XXI

PLATE XIX

All drawings were made with the aid of a projection apparatus magnifying 600 times. All drawings show xylem, cambium, and a few phloem cells.

FIG. 1.—Ash, March 28: xylem formed in 1924; no new xylem.

FIG. 2.—Ash, April 15: xylem formation begun.

FIG. 3.—Ash, April 20: portion between thick walled 1924 xylem and cambium layer is 1925 xylem.

FIG. 4.—Ash, April 27: all xylem shown was formed in 1925; thickening and lignification of cell walls begun.

FIG. 5.—Ash, May 14: all xylem shown except upper row or two of cells was formed in 1925; spring xylem cells formed.

FIG. 6.—Ash, June 13: portion of 1924 xylem shown at upper edge of drawing; walls thickening rapidly.

FIG. 7.—Ash, June 28: total 1925 growth up to date; most of summer xylem cells formed.

PLATE XX

FIG. 8.—Ash, July 15: only portion of 1925 xylem adjacent to cambium shown, rest mature; cell division proceeding more slowly.

FIG. 9.—Ash, August 1: section from one side of trunk showing cessation of xylem formation.

FIG. 10.—Ash, August 1: section from opposite side of trunk shown in fig. 9, showing that xylem formation had started again.

FIG. 11.—Ash, August 15: very slight cambial activity; practically all xylem fully lignified.

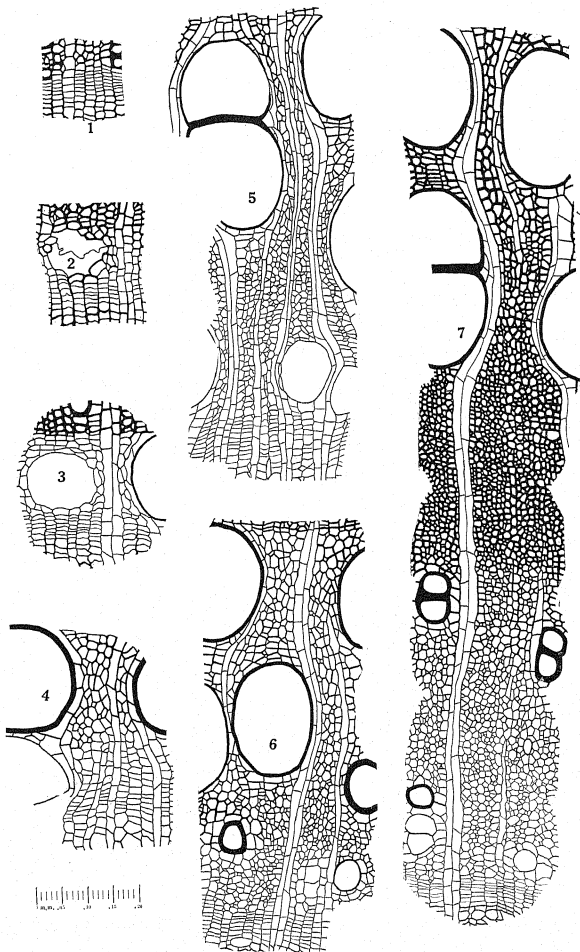
FIG. 12.—Ash, September 14: xylem fully lignified to cambium, and cell formation apparently ceased.

FIG. 13.—Soft maple, April 13: xylem formation not started.

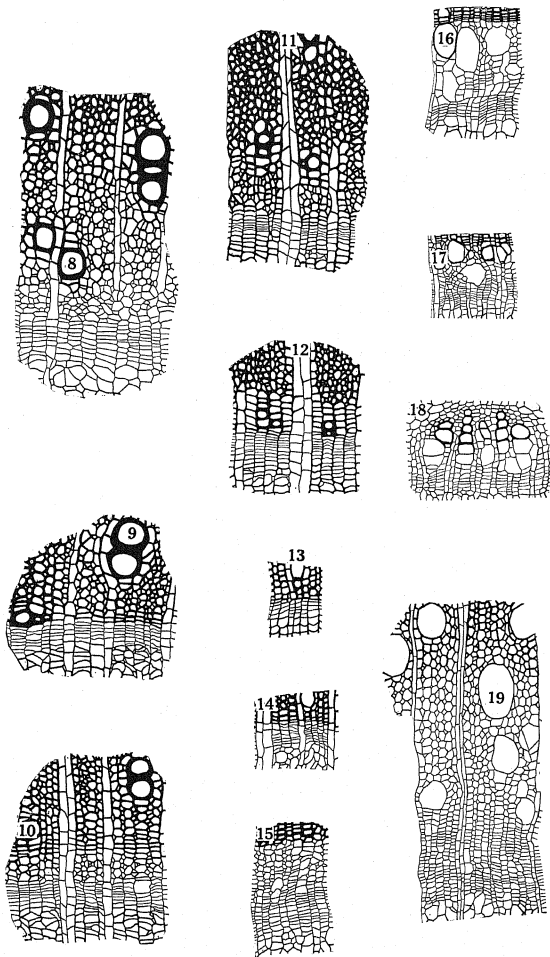
FIG. 14.—Soft maple, April 20: section from one side of trunk showing no or very slight cambial activity.

FIG. 15.—Soft maple, April 20: section from opposite side of trunk from which fig. 14 was taken showing vigorous xylem formation.

FIG. 16.—Soft maple, April 27: section from trunk at height of 18 feet showing vigorous xylem formation.









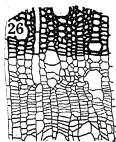
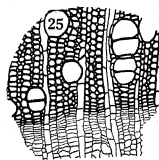
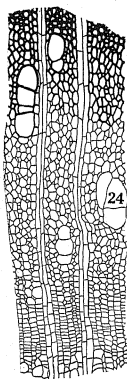
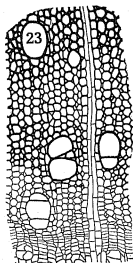
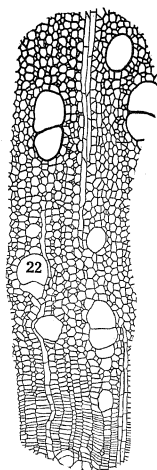
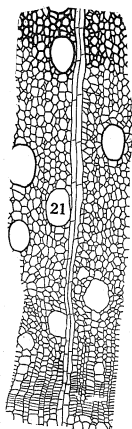
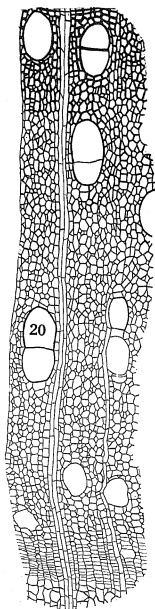




FIG. 17.—Soft maple, April 27: from 1-year-old twig.

FIG. 18.—Soft maple, April 27: 1925 twig, showing xylem and cambium of medium sized bundle.

FIG. 19.—Soft maple May 7: all xylem shown was formed in 1925; thickening and lignification of walls started.

PLATE XXI

FIG. 20.—Soft maple, May 27: all xylem shown except uppermost row or two was formed in 1925.

FIG. 21.—Soft maple, June 13: portion of 1925 xylem shown.

FIG. 22.—Soft maple, June 29: portion of 1925 xylem shown; xylem formation proceeding rapidly.

FIG. 23.—Soft maple, July 15: portion of 1925 xylem shown; xylem formation has decreased.

FIG. 24.—Soft maple, August 1: portion of 1925 xylem shown; xylem formation proceeding rapidly.

FIG. 25.—Soft maple, August 15: portion of 1925 xylem shown; appearance of new cells ceased; thickening and lignification complete to cambium.

FIG. 26.—Soft maple, September 14: formation of xylem cells started anew after apparently ceasing for season.

EFFECT OF LIGHT INTENSITY ON GROWTH OF SOY BEANS AND ITS RELATION TO THE AUTO-CATALYST THEORY OF GROWTH

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 357¹

HENRY W. POPP

(WITH EIGHT FIGURES)

Introduction

A previous study of the effect of various ranges of wave-lengths of the sun's spectrum on the growth of plants, carried out at the Boyce Thompson Institute, showed that when the Peking variety of soy beans was deprived of blue-violet rays the stems grew excessively long and twined, while plants grown under the same total light intensity but with all the rays of the sun's spectrum present gave no such response. The object of the present study was to determine whether similar effects could be produced by decreased light intensity alone, the plants receiving all rays of the sun's spectrum but at much lower intensities than were used in the previous experiment.

Materials and method

Six compartments, each 44×50 inches at the base and 40 inches high, were constructed inside a large greenhouse. These compartments were completely inclosed with cloth so as to give different degrees of light intensity, as indicated in table I. The light intensities were measured at different times during the day by means of a Macbeth illuminometer. The average temperature during the growth period in these compartments was 23° C. during the day and 19° at night. At no time did the temperature vary more than 1-3° C. in the different compartments. The temperature of compartment 6 was always about 1° lower than that of compartment 1.

¹ Joint contribution with the Department of Botany, Pennsylvania State College, no. 57. Published by permission of the Director of the Agricultural Experiment Station, Pennsylvania State College, no. 403.

Four varieties of soy beans were used, Peking, Mandarin, Biloxi, and Ito San. Three pots of each variety, containing four plants each, were placed in each compartment, and were so arranged as to eliminate variations resulting from different positions within the compartments. All plants were grown in 6-inch pots in the same batch of soil, a well fertilized dark sandy loam. All other conditions were kept as uniform as possible. The seeds were all sown on July 11. Weekly measurements of height were made and general observations taken on time of flowering, thickness of stems, leaf development,

TABLE I

COMPARTMENT NO.	COVERING	AVERAGE LIGHT INTENSITY FOOT-CANDLES (CLEAR DAYS)	APPROXIMATE PERCENTAGE TRANSMISSION OF COVERINGS (OUT- SIDE INTENSITY = 100 PER CENT)
1.....	None	4285	66.00
2.....	One layer white cheesecloth	1536	23.00
3.....	Two layers white cheesecloth	560	8.60
4.....	Three layers white cheesecloth	390	6.00
5.....	Four layers white cheesecloth	250	3.80
6.....	Two layers white cheesecloth plus one layer black calico	26	0.38

color of leaves, vigor, etc. The plants in all compartments were supported by means of bamboo canes after two weeks of growth. The experiment was continued for seven weeks.

General results

The average measurements of length of stems are given in table II and the curves of growth obtained from these data in figs. 5 to 8. Figs. 1 to 4 are photographs of the different varieties taken after six weeks of growth. The plants of compartment 6 are not shown in these photographs since they were all dead at this time.

In the early stages of growth (1-2 weeks) the rate of stem elongation in all varieties was greatest in the darkest compartment (no. 6) and least in the brightest compartment (no. 1), being in general inversely proportional to the light intensity. Gradually the majority of the plants in compartment 5 gained in rate over those of compartment 6; then those of compartment 4 gained over those of compart-

ment 5; and finally the plants of compartment 3 attained the most rapid rate of the series, with the final result that the tallest plants occurred generally in compartment 3, which had an intermediate light intensity.

TABLE II

AVERAGE HEIGHT OF PLANTS IN CM.; AVERAGE OF 12 PLANTS OF EACH VARIETY IN EACH COMPARTMENT

VARIETY	COMPARTMENT NO.						WEEKS FROM TIME OF PLANTING
	1	2	3	4	5	6	
Peking.....	3.61	4.40	5.18	4.66	6.72	11.02	1
Mandarin.....	6.40	6.32	7.35	8.90	10.61	18.75	
Biloxi.....	4.98	5.81	5.94	7.95	9.38	13.06	
Ito San.....	6.60	7.20	8.40	9.96	11.91	17.91	
Peking.....	8.78	10.86	15.23	16.68	17.54	26.00	2
Mandarin.....	13.14	13.78	17.50	23.29	24.60	37.35	
Biloxi.....	13.30	16.10	21.48	19.56	24.48	36.95	
Ito San.....	14.56	17.52	20.20	24.63	26.20	29.02	
Peking.....	11.90	16.16	22.33	24.61	26.28	29.07	3
Mandarin.....	17.92	19.60	25.90	34.75	34.55	38.59	
Biloxi.....	17.37	24.21	34.84	28.80	40.11	44.73	
Ito San.....	21.25	26.75	32.64	38.54	39.70	30.14	
Peking.....	19.50	25.93	40.09	39.22	34.21	29.50	4
Mandarin.....	27.65	26.19	38.75	46.37	40.64	Dead	
Biloxi.....	25.35	31.94	54.43	41.08	52.40	48.15	
Ito San.....	29.70	35.46	50.00	51.65	48.58	Dead	
Peking.....	31.58	40.85	64.83	56.31	47.78	Dead	5
Mandarin.....	35.90	34.80	53.80	56.48	43.75	Dead	
Biloxi.....	35.79	45.00	80.94	57.01	64.06	Dead	
Ito San.....	42.60	51.30	77.49	69.00	54.45	Dead	
Peking.....	42.35	59.93	90.32	73.83	57.07	Dead	6
Mandarin.....	41.21	37.66	57.70	58.69	44.22	Dead	
Biloxi.....	46.81	63.32	98.55	72.77	78.31	Dead	
Ito San.....	42.63	65.32	89.73	76.27	58.12	Dead	
Peking.....	51.08	76.79	104.40	82.50	62.10	Dead	7
Mandarin.....	41.37	37.68	58.18	58.74	45.16	Dead	
Biloxi.....	52.98	71.99	111.94	82.80	92.72	Dead	
Ito San.....	57.06	70.04	91.52	77.18	59.50	Dead	

There is apparently an optimum light intensity for stem elongation, which in this case was around 500 foot-candles. The falling off in rate of elongation in compartments 4 to 6 probably resulted from the inability of the plants to synthesize sufficient food to maintain rapid growth. This is evidenced by the fact that the plants of compart-

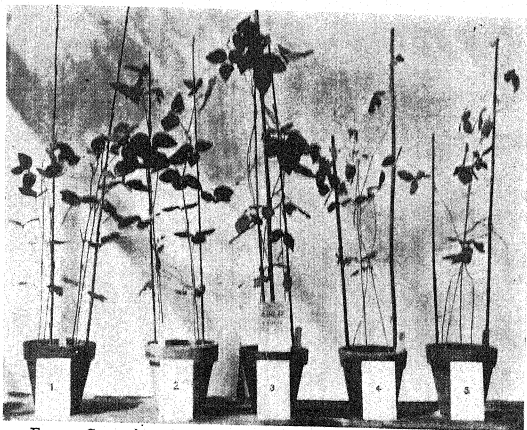


FIG. 1.—Comparison of Peking soy beans in compartments 1 to 5 (numbered from left to right).

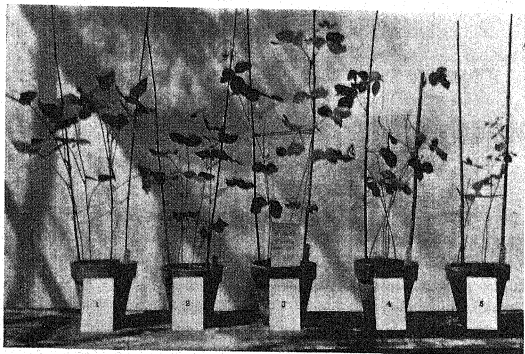


FIG. 2.—Comparison of Mandarin soy beans in compartments 1 to 5 (numbered from left to right).

ment 6 died as soon as the food in the cotyledons was depleted; in fact, these plants were almost as completely etiolated as though they had been grown in total darkness. On the other hand, the lower stature in compartments 1 and 2, under higher light intensities, was always associated with greater thickness of stems and greater

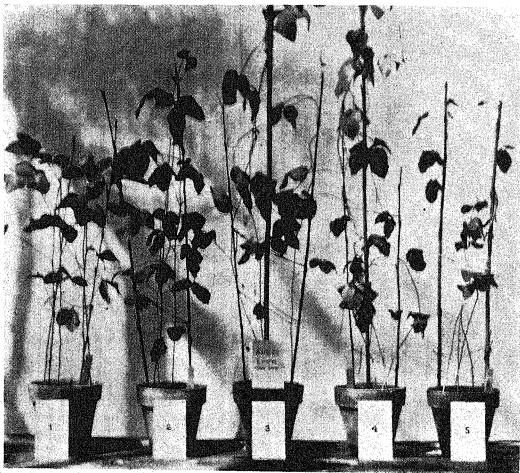


FIG. 3.—Comparison of Biloxi soy beans in compartments 1 to 5 (numbered from left to right).

size of leaves, showing that these plants had made more growth in weight than any of the others. In general the thickness and toughness of the stems were directly proportional to the light intensity, being greatest in compartment 1 and least in compartment 6. Practically all of the plants in compartments 2 to 6 were abnormally long stemmed.

There was very little difference in time of flowering in the first five compartments. The plants in each succeeding compartment be-

yond the first were about a day later in flowering than those of the preceding one. No flowering occurred in compartment 6, the plants having died before reaching that stage. Flowering began in the Mandarin variety during the fifth week, in the Ito San variety during the sixth week, and in the Peking variety during the seventh week. None

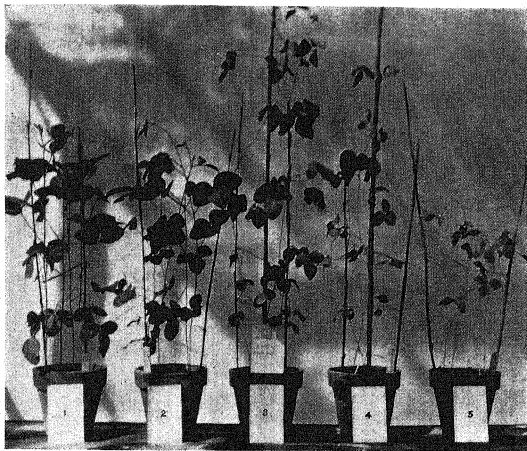


FIG. 4.—Comparison of Ito San soy beans in compartments 1 to 5 (numbered from left to right).

of the Biloxi plants had flowered by the time the experiment ended. The development of fruits decreased gradually from compartments 1 to 5. Practically no fruit developed in compartment 5.

Twining

Twining occurred in all varieties used, appearing first in compartment 5, next in compartment 4, then in compartment 3, and finally, although much less pronounced, in compartment 2. No twining occurred in any variety in compartments 1 and 6, which were

at the extremes of the series in light intensity. Apparently twining in soy beans is associated with stem length and thickness. It would appear from all studies reported and from this study that any condition that causes abnormal elongation, accompanied by decreased stem thickness or toughness, within certain limits will induce twining. The stem length necessary to induce twining is conditioned by the stem thickness, but there appears to be a minimum length of stem below which twining does not occur. In no case did any of the plants twine from the beginning of growth. The first indication of twining occurred in compartment 5 during the fourth week of growth, when the plants were 35-50 cm. high. No twining occurred in any plant before it was 35 cm. high. Twining did not occur in compartment 1, in spite of the fact that these plants attained a height of over 50 cm. and were supported in the same way as the other plants, but in this case the stems were thick and strong. On the other hand, some of the Biloxi plants in compartment 6 reached a height of 48 cm. but did not twine. In this case the stems were extremely thin and weak. Most of the plants in this compartment, however, never attained the height at which twining began in the other compartments. From the fact that the thinner stemmed plants of compartments 3 to 5 twined before the thicker stemmed plants of compartment 2, it seems that the thicker the stem, the greater must be the period of growth before twining begins.

These results seem to confirm the postulate of KLEBS, that a plant is endowed with a group of potentialities, and whether these assert themselves or not depends upon the environmental conditions under which the plants are grown. In soy beans one of these inherent tendencies seems to be toward twining. Whether twining is obtained or not depends partly upon the light conditions. The optimum for twining, however, is not the optimum for general vigor. That twining is not generally caused in plants in this way is shown by the fact that in studies of the effect of various ranges of wave-length of the sun's spectrum on the growth of plants, many other species (tomato, petunia, four o'clocks, coleus, etc.) grew to unusual length but did not twine under conditions that caused uniform twining in soy beans. In other words, there does not seem to be a latent factor for twining present in these other plants.

Rate of stem elongation and the autocatalyst theory

This study has brought out some interesting points in connection with the autocatalytic growth theory as advanced by ROBERTSON² and others. This theory, based on the observation that the rate of growth of an organism, when expressed in terms of height, dry weight, etc., gives a curve that is similar to the curve of a monomolecular autocatalytic reaction, assumes that growth is a catalytic process, and that the organism may be looked upon as the end product resulting from the action of an autocatalyst on a substrate. The actual configuration of the curve of growth, therefore, is thought to be caused by the activities of an autocatalyst, which, however, has not yet been identified, although several hypothetical substances have been suspected by different advocates of the theory. In a preliminary paper, MURNEEK³ recently questions the validity of the autocatalyst theory, and gives some evidence to show that growth rate in plants can be explained on the basis of correlation. According to him the "autostatic" or later phase of the growth curve of a plant is produced "by the effects of correlation of the fruit on the rate of growth of the main stem," while the "autokinetic" phase "may probably find its counterpart of explanation in the influence of correlation of roots on the stem."

In the present study, after the curves of growth in height were plotted, it was discovered that these curves for the plants in the first five compartments (figs. 5 to 8) were similar to the curve of a monomolecular autocatalytic reaction. The similarity was most pronounced in the curves of the plants of the first three compartments, which had the highest light intensities. These plants started out at a comparatively slow rate of growth; gradually increased in rate until a maximum was reached; and finally *in those plants that flowered* fell off again. In some cases there was a slight falling off in rate between the second and third week. The plants in compartment 6, however, which had the lowest light intensity of the series, started out at a more rapid rate, maintained a fairly high rate up to the end of the second week and then fell off rapidly, thereby giving only the first part of the curve given by the other plants. It was near the time

² ROBERTSON, T. B., The chemical basis of growth and senescence. London. 1923.

³ MURNEEK, A. E., BOT. GAZ. 79:329-333. 1925.

when the plants of compartment 6 died that the plants of the other compartments started on their most rapid rate of growth. It is

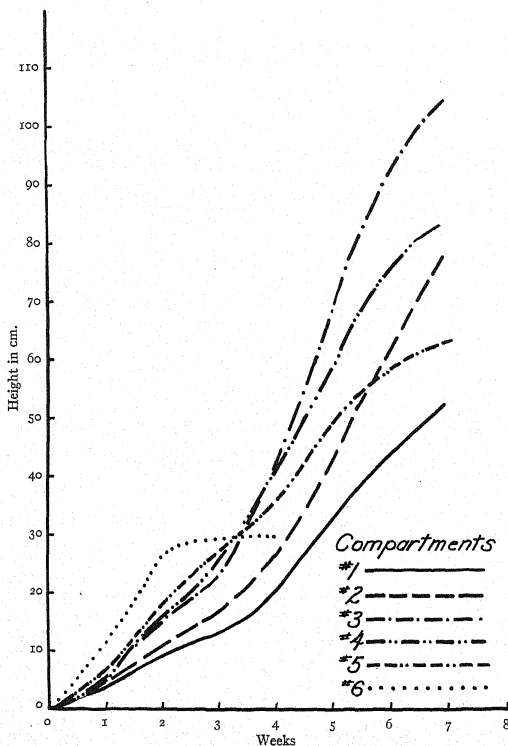


FIG. 5.—Height growth curves for Peking soy beans; plants started to flower at end of seventh week.

significant to note that this also was the time when the plants of the first five compartments began to develop mature green leaves, and therefore to manufacture enough food by photosynthesis to enable them to become independent of the food reserves in the cotyledons.

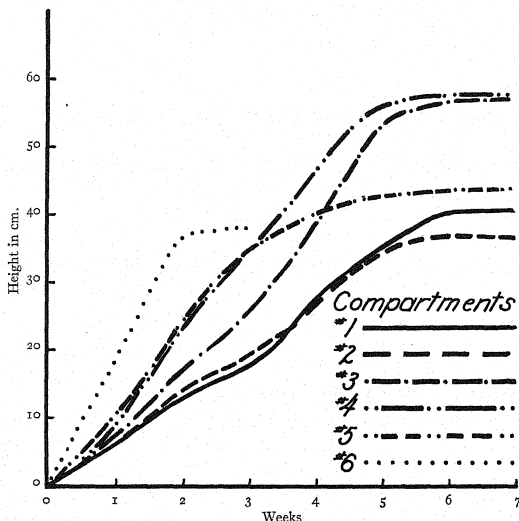


FIG. 6.—Height growth curves for Mandarin soy beans; plants started to flower at end of fifth week.

The plants of compartment 6, on the other hand, being very pale and almost completely etiolated, were unable to carry on photosynthesis to a sufficient degree to keep them alive after the reserve food of the cotyledons was depleted. The growth these plants made, therefore, represents the maximum growth that could be made from food stored in the seed, and the curve of this growth corresponds to the first part of the curves of the other plants. The configuration

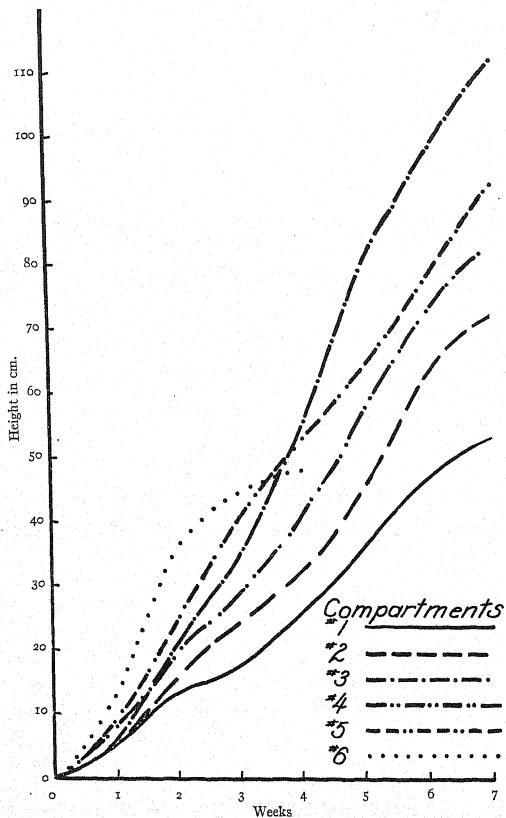


FIG. 7.—Height growth curves for Biloxi soy beans; no indication of flowering at end of seven weeks.

of the first part of these curves (autokinetic phase), therefore, appears to be associated with the initiation and development of independence in the seedling, or with the gradual ascendency of growth

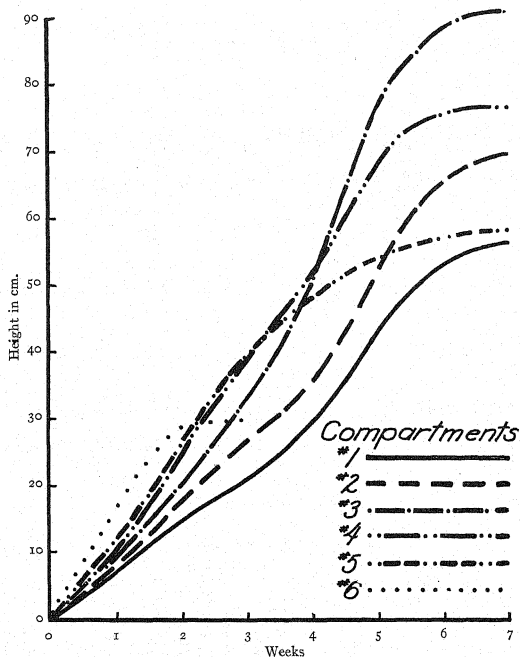


FIG. 8.—Height growth curves for Ito San soy beans; plants started to flower at end of sixth week.

resulting from food manufactured by photosynthesis over that resulting from food stored in the cotyledons. Certainly during the first part of the autokinetic phase the food is supplied by the reserves in

the seed. Since the supply is relatively large at first, the rate of growth soon becomes rapid. As the supply decreases and as more and more new tissues are made which must be supplied with food, the rate falls off unless a new and adequate supply is made by photosynthesis; but if there is sufficient light and leaf surface for photosynthesis, growth proceeds at a more rapid rate. If we examine the latter half of the curves we find that this rapid rate of growth continues so long as the plant does not flower. In all varieties that flowered, growth in length practically ceased when flowering began, while in the Biloxi variety, which did not flower, rapid growth continued. In those cases where flowering occurred, the food was apparently carried to the developing fruits and not to the growing points of the stem, and hence growth in length ceased. The falling off in rate in the "autostatic" phase of the curve, therefore, is caused by the development of flowers and fruit. This last point is exactly in accord with the facts MURNEEK pointed out.

While this study does not in any way prove the absence of an autocatalyst, yet it shows that it is possible to interpret the configuration of the curves of growth on a more tangible basis than to assume the presence of hypothetical substances.

Summary

The effect of different light intensities on the growth of soy beans was studied by growing four different varieties under six different light intensities, averaging 4285, 1536, 560, 390, 250, and 26 foot-candles respectively. The results were as follows:

1. The lower the light intensity, the more rapid was the rate of stem elongation during the initial period of growth.
2. The greatest general height was attained by plants under a light intensity averaging 560 foot-candles, and the lowest under an average intensity of 26 foot-candles.
3. The thickness of the stems was directly proportional to the light intensity, being greatest under 4285 foot-candles and least under 26 foot-candles.
4. In general all of the plants were unusually long stemmed. Those receiving the greatest amount of light were the most vigorous, produced the best leaves and color, and the best fruit. There was a

gradual decrease in vigor, etc., with decreasing light intensity. Plants grown under 26 foot-candles were completely etiolated and died within three to four weeks.

5. Twining occurred in all plants under light intensities between 250 and 1536 foot-candles, but not under 4285 foot-candles nor under 26 foot-candles. Soy beans apparently have a latent factor for twining which is associated with stem length and thickness. Thick stems require a greater length for twining than thin ones, but in no case did twining occur in a stem less than 35 cm. high.

6. The curves of growth in length of all plants except those in the darkest compartment followed the general curve of a monomolecular autocatalytic reaction. The autokinetic phase of the curves appeared to be associated with the initiation and development of independence in the seedling brought about by the ascendancy of photosynthesis, while the falling off in rate during the latter part of the autostatic phase was caused by the development of flowering and fruiting.

The writer desires to acknowledge his indebtedness to Professor C. A. SHULL for his interest and helpful suggestions in this work.

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EFFECT OF CERTAIN DEFICIENCIES ON NITROGEN METABOLISM OF PLANTS

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Introduction

Very little is known concerning protein synthesis in plants. The intermediate steps involved in the building of these compounds from the simple plant nutrients have never been satisfactorily worked out.

There is evidence that some of the mineral elements recognized as essential for plant growth play important rôles in certain stages of the protein synthesis. For example, SCRUTI (5) has attributed to phosphorus an important rôle in higher plants in the formation of the amino acids; and recently, KRAYBILL (4) has obtained some interesting results in an extensive study of the relation of phosphorus to the nitrogen metabolism of the tomato. Limiting the available quantities of such elements should throw the ordinary leaf machinery out of gear, permitting certain intermediate products of protein synthesis to accumulate, while other substances would cease to be formed, or at least would be produced in only abnormally small amounts. If lack of a certain mineral element, as calcium, would retard or stop the protein synthesis at a different stage from that caused by the lack of some other essential element, as potassium, then intermediate products of varying degrees of complexity might be determined; also some definite functions of these essential elements would be established.

In this investigation the effects of magnesium, potassium, and calcium deficiency on the composition of certain plants were studied. The analyses were compared with analyses of control plants grown side by side with the deficient plants, but supplied with all the essential nutrient elements.

Experimental procedure

Soy beans (Peking) and pumpkins (Connecticut Field) were the plants selected for the experiments, because they were found to make a rather rapid growth and to thrive moderately well in the green-

house. Navy beans were tried at first, but when grown in sand cultures they were found to be very susceptible to a bacterial wilt, apparently carried by the seed. The plants were grown in trays filled with washed quartz sand. The bottoms of the trays were brushed over on the inside with melted paraffin, the moist sand put in, and the seeds planted in rows three inches apart and one-half inch apart in the rows. Subsequent watering was carried out with the diluted nutrient solutions described later. As required, the same quantities were added to each tray in the same way.

To take into account the effect of greenhouse conditions and other factors that could not be definitely controlled, the trays were alternated with trays of control plants on the greenhouse bench. The development and composition of the deficient plants and of the control plants were then compared. Since all external conditions were approximately the same in the cases of the plants compared, except that one element was deficient, the differences observed were assumed to be due to this deficiency.

Five days after the seedlings appeared above ground, the cotyledons were removed in order that the effects of the deficiency might appear sooner. Thirty days after this (35 days above ground) the plants were sampled. Definite numbers of average plants were removed from the deficient and control trays and stripped of their leaves (petioles removed). These leaf samples were then weighed and preserved in 80 per cent alcohol, with the addition of calcium carbonate, as later described. The stems, from the roots to the point of attachment of the cotyledons, were preserved similarly as separate samples.

The nutrient solutions used were similar to those employed by DICKSON (2), except that they were recalculated to an absolute deficiency of the element in question, instead of merely reducing it to one-tenth of that in the normal solution used. This is essentially Knop's solution, diluted one to ten, with corresponding deficient solutions containing exactly the same physiological balance of all the other essential elements except the one that is lacking. The dilution is necessary to take care of the accumulation of salts in sand cultures watered with nutrient solutions. Chemically pure reagents were used. Calcium nitrate and magnesium sulphate solutions were pre-

pared by making approximate solutions from the hydrates, determining the concentration of calcium and magnesium in each by analysis, and then making appropriate dilutions. A block tin condenser was used in the preparation of the distilled water, and solutions were stored in seasoned alkali treated flint glass bottles. Stock solutions of the various salts were prepared which served during the whole experiment. The hydrogen-ion concentrations of the diluted solutions, as actually applied to the plants, varied between 4.5 and 5.0 P_H . The osmotic pressure of each solution was approximately 0.0151; that of the calcium deficient solution was very slightly

TABLE I
CONCENTRATIONS OF SALTS IN NUTRIENT SOLUTIONS (GM. PER LITER)

	NORMAL	Mg DEFICIENT	K DEFICIENT	Ca DEFICIENT
Calcium nitrate.....	0.533	0.533	0.533
Potassium nitrate.....	0.133	0.133	0.133
Potassium phosphate.....	0.133	0.133	0.133
Magnesium sulphate.....	0.133	0.133	0.133
Sodium chloride.....	0.067	0.070	0.063
Sodium nitrate.....	0.111	0.552
Sodium sulphate.....	0.1576
Sodium phosphate.....	0.117

higher. The concentrations of the diluted solutions are given in table I. Two and five-tenths cc. M-100 ferric chloride solution was added to each liter of nutrient solution just before applying to the plants.

When the plants had grown 35 days above ground, they were sampled as described. Sufficient data were recorded so that the composition of the plants could be expressed in several ways (fresh weight, dry weight, and per plant). Cotyledons, leaves, and stems were sampled separately. The sample, 50-100 gm. weighed accurately to one decigram, was cut in small pieces with a pair of scissors. Five-tenths of a gram of calcium carbonate (ppt. chalk) was sprinkled over it to neutralize the acidity of the solution, which would cause the inversion of sucrose and other changes; sufficient 95 per cent aldehyde free alcohol and water was added to bring the final concentration of alcohol to 80 per cent. The mixture was heated rapidly to boiling on the steam bath to stop enzyme action, and set

aside, carefully covered, for a day or longer. The liquid was then decanted off, filtered through a hardened filter, and the residue dried at 105° and pulverized. The powdered material was placed in a Vivian extractor and extracted with some of the alcoholic filtrate until the percolate was colorless. The volume of the total extract was made up to 1 liter with 80 per cent alcohol, and aliquots of this solution were used in the determination of the various soluble nitrogen and carbohydrate compounds, as follows:

Chemical analyses of samples

SUBSTANCE DETERMINED

- | | | |
|-----------------------|---------------------|------------------------|
| 1. Insoluble residue | 5. Nitrate nitrogen | 9. Amino acid nitrogen |
| 2. Solids in extract | 6. Nitrites | 10. Reducing sugars |
| 3. Insoluble nitrogen | 7. Ammonia nitrogen | 11. Sucrose |
| 4. Soluble nitrogen | 8. Amide nitrogen | 12. Starch |

METHODS

1. **INSOLUBLE RESIDUE.**—The material remaining from the final alcoholic extraction of the leaf tissue was dried at 100° C. and corrected for the added calcium carbonate.

2. **SOLIDS IN EXTRACT.**—Fifty cc. aliquots of the alcoholic extract were evaporated and the residue dried at 100° C.

3. **INSOLUBLE NITROGEN.**—The determination was made by the Kjeldahl process, on 1 gm. samples of the dried residue from the extraction. These were ground to pass through an 80 mesh sieve.

4. **SOLUBLE NITROGEN OF EXTRACT.**—Total nitrogen, including nitrates (preliminary reduction with Devarda's alloy), was determined, using 50 cc. aliquots of the alcoholic extract.

5. **NITRATE NITROGEN.**—This was determined by the phenol di-sulphonic acid colorimetric method as modified by BURRELL and PHILLIPS (1).

6. **NITRITES.**—Aliquots of the alcoholic extract were treated as in the nitrate determination, up to the point of being oxidized with sodium peroxide. At this stage they were neutralized with sodium hydroxide solution, and nitrites were determined colorimetrically, using the alpha naphthyl amine-sulphanilic acid reagent.

7, 8. **AMMONIA AND AMIDE NITROGEN.**—The Van Slyke Cullen urea apparatus was used, the ammonia being liberated by 52 per

cent potassium carbonate solution. Aeration was carried on for one hour into 0.02 N acid. Blanks were run simultaneously and the ammonia determined by titration. For amide nitrogen, the sample was subjected to a preliminary amide hydrolysis, and the total ammonia nitrogen was then found as previously described. Subtracting the ammonia nitrogen previously found present as such, gave the amide nitrogen.

9. AMINO ACID NITROGEN.—This was determined by the Van Slyke method, using the micro apparatus.

10. REDUCING SUGARS.—Picrate reduction and colorimetric estimation were used. The sample was prepared by the method recommended by THOMAS and DUTCHER (7). Analysis of the clear filtrate, however, was carried out by the method as described by WILLAMAN and DAVISON (8), making use also of their correction tables.

11. SUCROSE.—Part of the clarified filtrate from "reducing sugars" was treated by the standard Herzfeld procedure, and a determination of total sugars carried out by the method used for reducing sugars. Making use of the appropriate corrections in WILLAMAN and DAVISON's paper, sucrose was calculated.

12. STARCH.—Two gram samples of the dried extracted residue were subjected to starch determinations according to the method of THOMAS (6).

Discussion

MAGNESIUM DEFICIENCY.—Magnesium deficient plants were not noticeably different in appearance from the controls (table II). A very small supply of magnesium (largely derived from the cotyledons before they were removed) seemed sufficient for the usual development of the chlorophyll and growth of the plant for the 35-day periods used in these experiments. Some navy bean plants which were grown to the blossoming stage, however, did show a much decreased vigor and the leaves were lighter in color than those of the controls. There is not a great difference in chemical composition between the magnesium deficient and control plants. In general, the quantity of starch is slightly less in the magnesium deficient plants in both leaves and stems; soluble nitrogen is slightly greater in the leaves of the magnesium deficient plants and about the same in the stems; and insoluble nitrogen is consistently slightly less in both the

TABLE II
ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS
MAGNESIUM DEFICIENCY

TISSUE	AGE IN DAYS	NO. OF PLANTS USED	FRESH WEIGHT (GAL.)	PERCENTAGE											N-TREATS	
				In-soluble residue	Solids soluble & per cent alcohol	Dry matter	In-soluble nitrogen	Soluble nitrogen	Nitrate nitrogen	Ammonia nitrogen	Amide nitrogen	Amino acid nitrogen	Reducing sugars	Sucrose		Starch
Cotyledons	5	500	93.1	9.84	3.35	13.19	0.626	0.167	0.0016	0.0300	0.080	0.32	0.10	0.38	+
Normal leaves	35	200	106.9	11.92	3.76	15.68	0.553	0.141	0.021	0.0002	0.0082	0.031	0.24	0.36	0.34	+
Deficient leaves	35	200	100.55	11.18	3.24	14.42	0.470	0.163	0.024	0.0002	0.0032	0.042	0.21	0.24	0.49	+
Normal stems	35	200	60.9	10.59	1.80	12.39	0.189	0.097	0.039	0.0086	0.014	0.30	0.05	0.18	+
Deficient stems	35	200	52.0	10.39	1.85	12.24	0.172	0.093	0.039	0.0101	0.010	0.22	0.04	0.13	+

TABLE III
ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS
POTASSIUM DEFICIENCY

TISSUE	AGE IN PLANTS DAYS	No. OF PLANTS USED	FRESH WEIGHT GM.	PERCENTAGE											N-TREATMENT	
				In-soluble residue	Solids soluble per cent alcohol	Dry matter	In-soluble nitrogen	Soluble nitrogen	Nitrate nitrogen	Ammonia nitrogen	Amide nitrogen	Amino acid nitrogen	Re- ducing sugars	Sucrose		Starch
Cotyledons	5	550	100.0	8.80	2.94	11.74	0.532	0.153	0.0018	0.032	0.071	0.28	0.15	0.32	+
Normal leaves	35	200	111.0	12.07	2.93	15.00	0.489	0.125	0.020	0.0002	0.005	0.045	0.24	0.28	0.30	+
Deficient leaves	35	200	100.6	12.22	3.40	15.68	0.442	0.147	0.033	0.0003	0.008	0.062	0.20	0.30	0.52	+
Normal stems	35	200	81.2	12.31	1.86	14.17	0.233	0.078	0.035	0.013	0.022	0.26	0.06	0.24	+
Deficient stems	35	200	69.6	11.07	2.15	13.22	0.167	0.083	0.028	0.015	0.025	0.15	0.07	0.14	+

TABLE IV
ANALYSES OF NORMAL AND DEFICIENT SOY BEAN TISSUES; FRESH WEIGHT BASIS
CALCIUM DEFICIENCY

TISSUE	AGE IN DAYS	No. OF PLANTS USED	FRESH WEIGHT (GAL.)	PERCENTAGE											Nr. TRIES	
				In-soluble residue	Solids soluble per cent alcohol	Dry matter	In-soluble nitrogen	Soluble nitrogen	Nitrate nitrogen	Ammonia nitrogen	Amide nitrogen	Amino acid nitrogen	Re- ducing sugars	Sucrose		Starch
Cotyledons	5	560	100.0	9.08	2.94	12.02	0.588	0.158	0.008	0.0022	0.040	0.083	0.35	0.12	0.21	+
Normal leaves	35	184	100.0	11.54	3.81	15.35	0.527	0.131	0.026	0.0008	0.007	0.046	0.28	0.33	0.31	+
Deficient leaves	35	252	100.0	11.83	4.28	16.11	0.344	0.108	0.049	0.0006	0.010	0.029	0.31	0.35	0.33	+
Normal stems	35	184	67.5	12.03	1.87	13.90	0.212	0.077	0.031	0.0004	0.011	0.019	0.21	0.08	0.27	+
Deficient stems	35	252	52.0	9.69	2.46	12.12	0.142	0.061	0.036	0.0005	0.006	0.013	0.23	0.13	0.15	+

stems and leaves of the magnesium deficient plants. There are many other slight variations in the quantities of the various substances determined, but these are quite apparent from the table. They are hardly sufficiently marked to justify any hypotheses to explain them until more data of this kind have been accumulated. Nodules were not developed on the roots of the soy beans in any of the experiments.

POTASSIUM DEFICIENCY.—

Plants grown under a deficiency of potassium differed considerably in physical appearance from the controls (table III). The leaves and stems were a different shade of green, and many of the leaves were blotched with lighter shades. Over 50 per cent of the potassium deficient pumpkin plants died from a bacterial infection during the seedling stage; none of the control plants grown side by side with these were so affected. The most noticeable difference in chemical composition between potassium deficient and control plants is the accumulation of starch in the leaves of the former. In the stems, how-

ever, starch is less with the potassium deficient plants than with the controls. HARTWELL (3) has also observed a similar condition, while WIMMER (9) found a failure to produce sugar and starch with potassium deficient plants, but this may vary with the stage of development. The percentage of dry matter is slightly higher in the potassium deficient than in control leaves. Insoluble nitrogen is less in all cases in both the stems and leaves of the potassium deficient plants; soluble nitrogen is greater; and amino acid nitrogen is especially high as compared with the quantity present in control plants. Potassium seems to have some function in the translocation and utilization of starch, and to be of especial importance in the formation of proteins from carbohydrates and amino acids.

CALCIUM DEFICIENCY.—Calcium deficient plants differed more markedly from the controls than did the potassium or magnesium deficient plants (table IV). The calcium deficient plants were smaller, of a yellow color, and showed less root development than the controls. When the sand in the trays had dried slightly, the roots of the calcium deficient plants could be pulled up with ease, while the control plants could not. The roots of the calcium deficient plants were noticeably brownish in color, especially on drying, and many were losing their epidermis. ECKERSON noted a similar condition in plants grown in water cultures deficient in calcium, and explained it by assuming that insoluble calcium pectate was normally present, but that with the calcium deficient plants the calcium was replaced by magnesium and other ions to form soluble pectates. The most striking feature of the chemical composition is the accumulation of nitrate in the leaves of the calcium deficient plants. Insoluble nitrogen is considerably less and amino acid nitrogen is less in both the leaves and stems of the calcium deficient plants. Calcium may play some important rôle in nitrate reduction, although, as previously stated, caution must be observed against drawing too sweeping conclusions until more data can be accumulated.

Conclusions

The use of deficient nutrient solutions for studying processes concerned in plant metabolism has been shown to have considerable possibilities. In these preliminary experiments the idea suggested at

the beginning of this paper was confirmed, namely, that certain elements play important rôles in different stages of synthesis occurring in plants. Also, when a certain essential element is markedly lacking, a process, as protein synthesis, may be greatly retarded at a certain stage, so that an abnormal accumulation of some intermediate product results. The products characteristic of the later stages of the synthesis are formed in abnormally small quantities.

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CARPELOID STAMENS OF *PODOPHYLLUM PELTATUM*

M. LOUISE SAWYER

(WITH FIVE FIGURES)

During the spring of 1919, on bringing into the botanical laboratory of Grinnell College, Iowa, a large collection of *Podophyllum peltatum*, taken in a country pasture and intended for class study, the writer found a number of abnormal flowers. These seemed to be sufficiently interesting to be worth investigating.

In these flowers (fig. 1) the pistil was quite normal and the floral envelopes practically so, although showing a slight tendency to have more than the usual number of petals. The unusual parts were the stamens. These organs were greatly enlarged at the apex by the occurrence, at the end of an expanded elongation of the connective, of a well developed stigmatic surface, closely resembling that borne normally on the pistil. These stamens with their terminating stigmas were of varying forms, and were found to bear not only pollen sacs containing pollen grains, but most of them also bore ovule-like structures of varying sizes and shapes. On many of the stamens the connective expansion was more or less like an open fan in shape, and the thickly convoluted margin was stigmatic (fig. 2). The ovules of these stamens were usually borne on the lower part of the expanded portion, not far from the pollen sacs. Other stamens bore disk-shaped enlargements with the stigmatic convolution surrounding the entire disk, except for an occasional interruption in the lower part near the pollen sacs. Often the spot not occupied by stigmatic tissue bore an ovule. Again, some of the stamens gave rise to several hornlike projections, each with stigmatic tissue at its tip. The most interesting variation in the form of the connective expansion was found on a few stamens which ended in a hoodlike structure that nearly inclosed a cavity. This condition is suggestive of an ovary. Such a stamen is discernible at the upper part of the stamen whorl in fig. 1. The ovules on these structures were located near the opening of the cavity, and the stigmatic surface was confined to the margin of the

tissue which surrounded the lacuna. Some of the stamens of this type lacked pollen sacs (fig. 3).

Ovuliferous stamens similar to those here reported have long been known. WORSDELL¹ figures, somewhat diagrammatically, a section throughout the stamens of *Papaver rhalis* var. *commutatum*, showing a proximity of pollen sacs and ovule-like



FIG. 1.—*Podophyllum* flower with abnormal stamens.

structures comparable with that found in this material of *Podophyllum*, but, so far as the writer knows, such reports do not include findings on the internal structure of the unusual ovules. This investigation was undertaken for the purpose of determining whether these bodies had the internal structure of ovules.

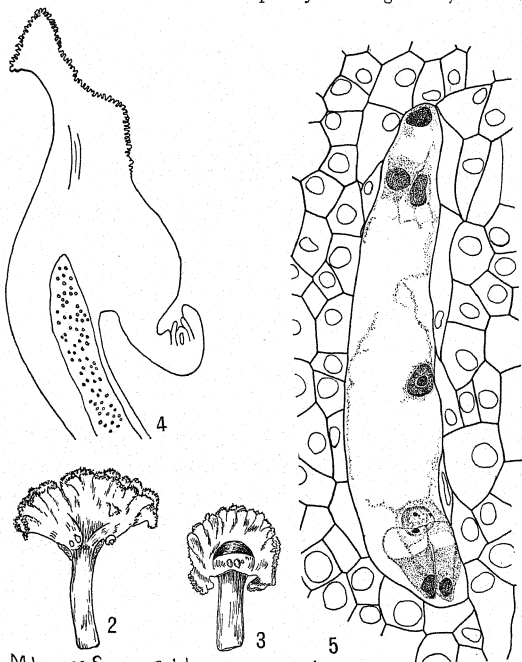
The material was fixed in a modification of Fleming's stronger solution and imbedded in paraffin. Serial sections were made and stained in Haidenhain's iron-haematoxylin.

Sections were obtained showing the relative positions of stigmatic tissue, ovules, and pollen sacs with the pollen grains (fig. 4). The pollen grains have the usual structure. They contain a recognizable generative cell and a tube nucleus, and appear wholly normal. Further, the ovule-like bodies were found to have the essential structure of ovules, but differed somewhat widely in the stage of development which they had attained, ranging from those showing a nucellus but partly enveloped by the integuments, to ovules with fully organized embryo sacs.

Some ovules show the nucellus containing an embryo sac and enveloped by the inner integument, the outer integument represent-

¹ WORSDELL, W. C., Principles of plant teratology. Vol. II. pp. 185-193, 1915.

ed only by an irregular collar at the base of the ovule. In some there is a nucellus more or less enveloped by the integuments, but con-



M. Louise Sawyer. del.

FIGS. 2-5.—fig. 2, one of the unusual stamens, bearing pollen sacs, ovules, and stigmatic tissue; fig 3, one of the unusual stamens, terminated by a hoodlike expansion which forms a nearly closed ovary-like cavity, with ovules borne near the opening; fig. 4, section through one of the unusual stamens showing stigmatic surface, ovule, and pollen sac containing pollen grains; fig. 5, mature embryo sac from an ovule borne on one of the unusual stamens.

taining no embryo sac. One series of sections gives an ovule with an embryo sac at the 2-nucleate stage. Another series of sections

shows an embryo sac in which the eight nuclei are evident and the polar nuclei are seen in contact, but antipodal nuclei have not organized cells, and at the micropylar end the egg apparatus is not developed, although the three cells are there. Still others reveal a mature embryo sac in which polar fusion seems to have occurred and the egg apparatus has the usual configuration (fig. 5).

Here, then, are sporophylls bearing both microsporangia and megasporangia. The microsporangia produce microspores which appear to become normal pollen grains, and the megasporules may give rise to normal embryo sacs, judging by the appearance of these structures in the sections. It would have been interesting to hand pollinate the unusual stigmas and attempt to secure ripe seeds on these carpeloid stamens. Unfortunately it was found that all of the unusual flowers in the sporting colony had been gathered. Had the writer been in the same locality another spring, an attempt to secure such results would have been undertaken, if the unusual flowers had been found a second season. BRONGNIART¹ reports a flower of *Pelemonium caeruleum* in which the stamens were represented by a circle of carpels around the central ovary, and by artificial fertilization he obtained fertile seeds from both the normal ovary and the surrounding metamorphosed stamens. It seems not unlikely that artificial pollination of the unusual *Podophyllum* material might have been followed by similar results.

There remains the interesting question whether there is a difference in the chromatic content of the usual egg and sperm in plants. If such a difference normally occurs, does it occur in the egg and sperm derived from these "bisexual" stamens, and at what point does the differentiation take place? Unfortunately the material contained no division stages, and suggests no answer to this problem, other than to indicate that if there are differences they must arise later than the differentiation of stamens and carpels, since here are stamens which give rise to both microspores and megasporules.

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[Accepted for publication August 5, 1925]

¹ BRONGNIART, A. T., Bull. Soc. Bot. France 8:453. 1844.

COSMOS BLAKEI, A NEW SPECIES FROM GUATEMALA
CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 358

EARL EDWARD SHERFF

(WITH PLATE XXII)

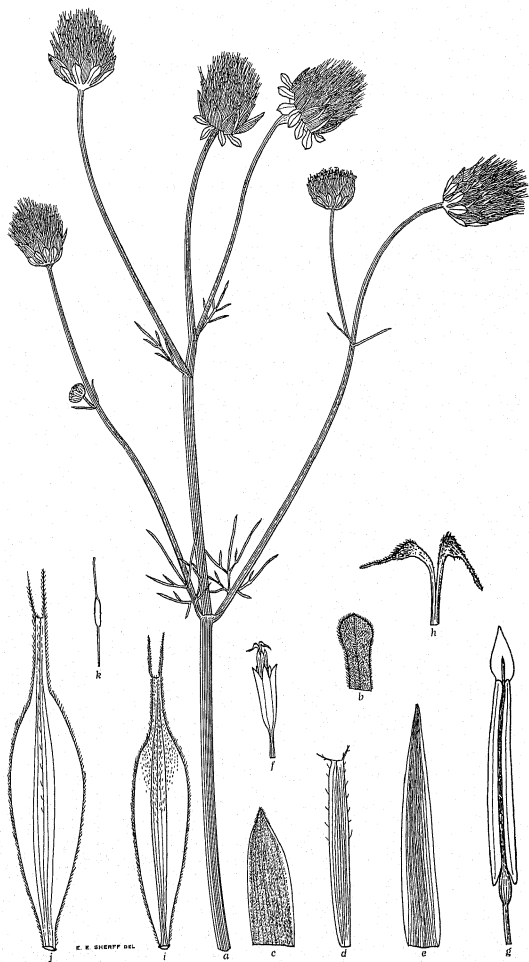
Among the interesting Guatemalan plants collected a half century or more ago by BERNOULLI and CARIO was their no. 1476. This was determined, with a mark of interrogation, as being a specimen of *Coreopsis*, and under that name the Kew specimen has lain until recently, apparently without special study. The general aspect of the plant offers a strange commingling of individual characters of such genera as *Bidens*, *Coreopsis*, and *Cosmos*. The fruiting heads, with their elongate, rostrate achenes, are particularly suggestive of *Cosmos*. The marginal wings of the achenes and also the upward direction of the hairs or setae both upon the achenial bodies and upon the achenial aristae, however, might indicate more of an affinity with *Coreopsis*. For several years I have hesitated to describe this species lest, because of its bizarre combination of characters, it might later appear to have been described previously in some genus unsuspected by me.

Some time ago Dr. S. F. BLAKE, Associate Botanist of the United States Bureau of Plant Industry, kindly made for me a very careful and extended supplementary study of the plant in question. He concluded it to be a new species. Furthermore, he concluded that it was best regarded, not as typifying a new genus, but as being an extreme form in the genus *Cosmos*. My own study leads to the same conclusion. With the species of *Cosmos* as known up to the time of BENTHAM and HOOKER's *Genera plantarum* (2:387. 1873) there appears a disagreement it is true, but some time later there was discovered the interesting *Cosmos exiguus* Gray (Proc. Amer. Acad. 22:429. 1887). In *C. exiguus*, which beyond all question is to be retained in *Cosmos*, the purple rays, the foliage (in larger specimens closely approaching that of *C. linearifolius* [Schz. Bip.] Hemsl.), and the long-rostrate

achenes are typical of *Cosmos* as formerly restricted by definition; but the dense achenial hispidity, the upward direction of both the corporal and the arisal setae upon the achenes, and the obcompressed, more or less margined or winged achenial bodies, all are at variance with the older definitions of *Cosmos*.

It is seen that the BERNOULLI and CARIO plant closely parallels *C. exiguus* in its more notable characters, and, with it, must be placed in *Cosmos*. For the trivial name I have chosen to commemorate the name of Dr. BLAKE, to whom it is a pleasure thus to acknowledge my indebtedness for his painstaking study of the herbarium material.

Cosmos Blakei, sp. nov.—Herba erecta, verisimiliter annua, infra glabra, supra pubescens, caule obscure tetragono, internodis longo, tantum moderate ramoso, ± 5 dm. alto (radice non visa). Folia tenuiter petiolata petiolis 1.5 cm. longis, petiolo adjecto ± 1 dm. longa, bipinnatisecta segmentis linearibus, acriter apiculatis, minute hispidis ac spinuloso-ciliatis, 0.5–1.5 mm. latis. Capitula ramos terminantia, robuste pedunculata pedunculis usque ad 1.2 dm. longis, radiata, pansa ad anthesin ± 2.5 cm. lata et ± 1 cm. alta. Involucrum pubescens, plus minusve campanulatum bracteis exterioribus spathulatis et supra saepe late rotundo-dilatatis, marginibus ciliatis apice aegre mucronatis, 4–6 mm. longis; interioribus multo maioribus, in sicco perspicue subflavis, late lanceolatis, ad apicem angustatum saepe minute glandulo-ciliatis, circ. 1 cm. longis. Flores ligulati circ. 8, tantum valde manci visi, in sicco atri vel obscure atro-purpurei, circ. 1.5 cm. longi; ovario lineari, membranaceo, sterili, in sicco nitido-albescente, marginibus et supra ad costam medianam erecto-hispido, corpore circ. 1 cm. longo, apice erecto-hispido et aegre aristato 1–2 aristis tenuibus sursum hispidis ± 0.5 mm. longis. Flores disci corolla tantum circ. 5 mm. longi, in sicco flavi. Achaenia (multa matura visa) valde obcompressa et infra circumambitu (alis latis inclusis) perspicue oblanceolata, faciebus atro-brunneis glaberrima vel supra et ad costam medianam erecto-setosa, marginibus straminea et setis spinulosis minute erecto-ciliata, supra cervicata cervice substraminea erecto-ciliata plerumque 3–7 mm. longa, tota longitudine (usque ad cervicis apicem) 1.4–2.4 cm., latitudine (alis inclusis) 2.7–3.4 mm.;





apice erecto-hispida et biaristata aristis erectis vel parce divergentibus, tenuibus, sursum hispidis, 1.5-2.5 mm. longis.

Type specimen: *Bernoulli* and *Cario* 1476
Retalhuleu, Guatemala, January 1871 (Herb. Kew).

CHICAGO NORMAL COLLEGE
CHICAGO, ILL.

[Accepted for publication February 24, 1926]

EXPLANATION OF PLATE XXII

Cosmos Blakei: *a*, fruiting specimen, uppermost part, $\times 0.6$; *b*, exterior involucre bract, $\times 3.6$; *c*, interior involucre bract, $\times 3.6$; *d*, ovary of ligulate floret, $\times 3.6$; *e*, palea, $\times 3.6$; *f*, disc floret, $\times 3.6$; *g*, stamen, $\times 30$; *h*, style-branches, $\times 22$; *i*, *j*, achenes, $\times 3.6$; *k*, transverse section of achene, $\times 7.2$; all from *Bernoulli* and *Cario* 1476, type in Herb. Kew.

BRIEFER ARTICLES

STRUCTURAL WEAKNESSES IN INTERSPECIFIC GRAFTS OF PYRUS

(WITH ONE FIGURE)

More than twenty years ago WAUGH¹ published a paper on the nature of the union between stock and scion, especially in hardwood grafting. I have been unable to find anything reported since that time that adds materially to our knowledge of the nature of the finer structural details of the union of grafted woody plants. Because of the obvious importance of a more thorough understanding of the nature of this phenomenon, an attempt was made to determine the characteristics of the unions of several interspecific grafts, with the hope that this information may aid in our understanding of the observed results of such grafts.

WAUGH has adequately treated the gross characters of the graft, finding (1) that the scion and the stock never grow together; (2) that the new layers of wood are produced in continuous layers in normal or successful unions; (3) that in imperfect unions the continuity of xylem is interrupted to a greater or less degree by the deposition of scar tissue, which makes the union mechanically weak; and (4) that this irregular structure is due to physiological incompatibility. His figures show the clear line of cleavage between stock and scion in both good and poor unions. He gives a single figure showing detail of union, a tangential section of a cherry-plum graft.

The studies reported here have confirmed the observations of WAUGH on the gross characters of the union, and the frequency of the occurrence of weak unions in some combinations due to the laying down of parenchyma at the line of union. There is often in addition a severe distortion of the vessels lying in the region of the union.

In the course of this study, a condition was found that seems not to have been reported earlier. This arrangement of tissue is illustrated in fig. 1, which shows a graft union of *Pyrus Malus* on *P. communis*, a combination usually incompatible. Instead of the layer of parenchyma that is usually found in weak unions, or associated with such a layer, there is bark tissue. This bark may roughly be divided into three parts, a corky

¹ WAUGH, F. A., The graft union. Mass. (Hatch) Agric. Exp. Sta. Tech. Bull. 2. 1904.

layer in the middle, of indeterminate origin, and a layer of bark on either side. Each of these two layers is characteristic of the bark of its species. In this case the layer shown at the top of the figure is apple and that at the bottom is pear.

A gross examination of the union from which this section was taken discloses the fact that the layer of bark extends nearly to the point at

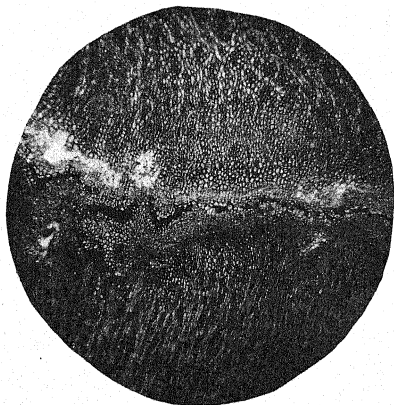


FIG. 1.—Tangential section of *Pyrus Malus* on *P. communis*, showing bark layers at line of union.

which the cambium layers of stock and scion were placed in contact at the time the graft was made. There has been enough movement of water and of nutrients through the relatively small amount of new tissue that is continuous to allow growth to proceed. It would appear that soon after growth had started, the region of the cambium which lay at the line of union ceased to function. As growth continued above and below, a layer of bark was laid down by the cambiums of both stock and scion. The result is a formation closely resembling in its final condition that described by MAC DANIELS² in narrow crotches. The layer of bark pinched between the

² MACDANIELS, L. H., The apple-tree crotch. Cornell Univ. Agric. Exp. Sta. Bull. 419. 1923.

xylem on either side forms a line of mechanical weakness. It is doubtful whether such a union would survive the second season, when a larger leaf surface would provide a greater resistance to wind movement and place a greater strain on the union.

The condition described has been observed in the following inter-specific grafts growing as one-year old trees in the nursery: *Pyrus Malus* on *P. serotina*, *P. Malus* on *P. communis*, *P. communis* on *P. Malus*, *P. communis* (var. Bartlett) on *Cydonia oblonga*, and *C. oblonga* on *P. serotina*. It occurred in both budded and grafted material.

It should be stated that there is a high degree of variability among the grafts of a given sort as to the frequency of the appearance of this condition and its extent in a given union. Thus, in the case of *P. Malus* on *P. serotina*, there are gradations from perfect unions to those having a mere shred of continuous xylem. Whether this variability is due to genetic differences in the seedling stocks or to the mechanical treatment and environmental conditions of the graft is not known.

Studies with species of *Prunus* have not been carried far enough to determine whether or not this condition exists in that genus. A condition that is somewhat similar, however, appears frequently in the case of *Prunus domestica* on *P. Persica*. In this case the activity of the cambium at the line of union is not entirely inhibited but greatly reduced. The result is a very gradual development of the condition, which does not cause a high degree of mechanical weakness, but which does interfere with translocation and may cause the death of the tree.—E. L. PROEBSTING, *University of California, Davis, Cal.*

[Accepted for publication February 26, 1926]

CURRENT LITERATURE

BOOK REVIEWS

Applied paleobotany

During recent years a number of paleobotanical publications were published in Germany which dealt with practical and theoretical conclusions that can be drawn from plant fossils.

In the series of volumes which G. GÜRICH publishes under the title of *Leitfossilien*, the third volume deals with the Carboniferous and Permian, and the plant fossils which serve as horizon markers in these formations.¹ This book represents the most modern textbook of paleobotany with special reference to the stratigraphic distribution of plant fossils. Previously only one attempt has been made to treat in a textbook the fossil plants from the point of view of their geologic distribution. This was done by Sir WILLIAM DAWSON in his geologic history of plants in 1880, but DAWSON's book is obsolete by this time. GOTHAN has rendered a great service in writing a book which can be taken out into the field and used for determinations as well as for reference. Although he considers almost exclusively the fossil plants of the European Carbon and Perm, a great deal of this information can be applied and utilized in America, because of the great similarity between the Upper Carboniferous and Permian of the two continents.

More theoretical than GOTHAN's book is another one which appeared as no. 8 of SOERGEL's *Fortschritte der Geologie und Palaeontologie*.² He tries to show what we may learn from the fossil plants to apply to biology, especially in adaptation. The book is more or less a collection of essays, and does not attempt to give a comprehensive treatment of the paleobiology of plants. Of special interest are the chapters on the periodicity of growth and on the climatic problems as they confront us in a study of fossil plants. Primarily the floras of the Upper Paleozoic are consulted.

A very interesting volume³ deals with the ability of plants to form rocks. PIA is familiar with this subject and has written a number of papers on calcareous algae. In the present volume he gives a detailed account of how strata can be formed through the activities of sulphur bacteria, iron bacteria, lime forming bacteria, unicellular algae, and organisms depositing lime or silica. Among the latter, of special interest are the diatoms. A chapter is devoted to

¹ GOTHAN, W., *Karbon und Perm-Pflanzen*. pp. viii+187. pls. 45. figs. 144. Berlin: Gebrüder Borntraeger. 1923. \$7.50.

² ———, *Palaeobiologische Betrachtungen über die fossile Pflanzenwelt*. pp. 178. Berlin: Gebrüder Borntraeger. 1924. \$2.25.

³ PIA, JULIUS, *Pflanzen als Gesteinsbildner*. pp. viii+355. figs. 166. Berlin: Gebrüder Borntraeger. 1926. \$5.00.

the rôle which unicellular plants play in the formation of coal and petroleum. A detailed account is rendered of the green algae, brown algae, and red algae, which form lime deposits. This part of the book includes also lime forming mosses and angiosperms. Nearly half of the book is devoted to coal and its formation, while the larger portion of the author's discussion deals with the paleozoic coals. He also considers the floras which have formed the lignite beds and the peat bogs of past and present.

PIA discusses in his book primarily the rôle which plants have played in former geologic periods in the formation of strata, but he always uses extensively for his explanations analogies with recent plants. Bibliographies at the end of chapters are helpful, although rather restricted to the more important publications. The illustrations are excellent and very instructive. The entire book is of great interest, not only to paleobotanists, but also to students of living plants.—A. C. NOÉ.

Results of biological research

Among the most welcome books nowadays are those presenting summaries or digests of recent researches in the various fields of knowledge. The first of a new series of volumes of this character comes from Germany. The field covered is biology in general, with special emphasis on the comparative physiology and psychology of animals, plant physiology, the mechanics of development, and theories of inheritance. There are four editors responsible for the series, FRISCH of Munich, GOLDSCHMIDT of Berlin, RUHLAND of Leipzig, and WINTERSTEIN of Rostock.⁴

The articles, of which there are six in the first volume, are by specialists in the field presented. Three of the articles, taking up five-sixths of the volume, are zoological or psychological. The botanical articles are on (1) the ascent of sap, by BACHMANN of Leipzig; the reactions of the plant cell to salts, by KAHN of Dorpat; and ammonia, nitrate, and nitrite as sources of nitrogen for the higher plants, by PRIANISCHNIKOW of Moscow.

Most of the article on the ascent of sap is devoted to DIXON's cohesion theory in the light of the more recent researches; a few pages are given to the condensation theory of BAKER, the polarity and enzyme theory of JANSE, and the curious pulsation theory of BOSE. A characteristic and welcome feature of this and the other articles of the volume is an ample citation of literature. This new series should be in every library which is consulted by research workers in the fields the series covers.—H. C. COWLES.

Citrus diseases

A book on citrus diseases and their control, by FAWCETT and LEE,⁵ will be the standard work in this field for some time to come, not only for those inter-

⁴FRISCH, K. VON, GOLDSCHMIDT, R., RUHLAND, W., and WINTERSTEIN, H., *Ergebnisse der Biologie*. Vol. I. 8vo. pp. viii+670. figs. 130. Berlin: Julius Springer. 36 marks; 38.40 marks bound.

⁵FAWCETT, H. S., and LEE, H. A., *Citrus diseases and their control*. 8vo. pp. xii+382. figs. 205 (15 in color). New York: McGraw-Hill Book Co. 1926. \$5.00.

ested in the scientific aspects of the subject, but also for those engaged in the production and marketing of citrus. Guided by the needs of growers and distributors, as well as by the requirements of investigators and teachers, the authors have of necessity been led to a more catholic concept and presentation of disease than is generally found in phytopathological literature. The authors squarely met the issue that a treatise on the pathology of a crop must be more than the applied mycology of that crop; consequently the volume takes up diseases due to animal and plant organisms as well as to non-living factors. The book is sound scientifically and pedagogically. The detailed description of the host of diseases that afflict all parts of the citrus plant in the grove and in the marketing processes is preceded by discussion of such fundamental topics as the species and varieties of citrus and their distribution in relation to disease; the normal structure and physiology of citrus as well as the relation of environmental factors to citrus culture and marketing, and to disease; and the nature and methods of control. The diseases are grouped for discussion on the basis of the organs affected, and under these headings useful keys are provided. The volume has all the earmarks of preparation by experts with firsthand knowledge of their subject matter. The literature is exhaustively treated, and the authors have taken commendable pains to give full credit to the many workers who have built up the structure of citrus knowledge. The volume is well printed and the illustrations, which are well chosen, are especially well done. The fifteen colored plates add materially to the usefulness and attractiveness of the book.—G. K. K. LINK.

The Cyanophyceae

A volume⁶ has just appeared in the series of monographs on the fresh water flora of Germany, Austria, and Switzerland, which will be welcome to all who are working with the blue-green algae, for the group is so cosmopolitan that the keys work very well with the Cyanophyceae of the Chicago region.

The first fifty pages deal with the cytology, morphology, phylogeny, and biology of the group, while the rest of the book is devoted to keys and taxonomic descriptions, all of which are in German. The illustrations are excellent and many of them are new.

The number of families is greater than in KIRCHNER's treatment in ENGLER and PRANTL's *Natürlichen Pflanzenfamilien*, and it would seem that there is a tendency to raise the rank of some forms, but this does not detract from the real value of the book. Fewer and fewer botanists are reading Latin fluently, and consequently the German keys will make the book more usable than those with Latin keys; besides, the book is up to date.—C. J. CHAMBERLAIN.

⁶ GEITLER, L., Die Süßwasser-Flora Deutschlands, Österreichs und der Schweiz. Heft 12. Cyanophyceae. 8vo. pp. viii+481. figs. 560. Jena: Gustav Fischer. 1925.

NOTES FOR STUDENTS

Taxonomic notes.—RILEY⁷ has published a revision of *Calycolpus* (Myrtaceae), a tropical American genus. He recognizes 12 species, 6 of which are described as new.

CRAIB,⁸ in continuation of his contributions to the flora of Siam, has described 28 new species, representing 16 genera, of which *Impatiens* includes 9.

KOBUSKI⁹ has revised *Priva*, a genus originally segregated from *Verbena*. It is a tropical genus, 9 species being endemic in the western tropics, and the other 2 (one of which is described as new) endemic in the eastern tropics. One species only extends into the United States. The author has also included a very complete list of collectors and stations.

EPLING,¹⁰ in his second contribution on the Labiatae of South America, has given a detailed account of the genus *Sphacele*, most of whose species are distributed along the western border. He recognizes 24 species, 3 of them being new.

GRENZEBACH¹¹ has published a revision of *Bouchea* (Verbenaceae), a small genus occurring chiefly in the Western Hemisphere, ranging from New Mexico to Bolivia. Only one species is known from the Eastern Hemisphere, occurring in Abyssinia. The 10 species include one that is new, and also 2 new varieties.

HONDA,¹² in continuation of his studies of the grasses of Japan, has presented 15 species in 8 genera, including 2 new species. The greatest changes occur in the varietal names, 18 new ones being proposed.

ITO and HOMMA¹³ have described a new genus (*Miyabella*) of Synchronitriaceae, including 2 species. It is a segregate from various genera of the family. The generic name is in honor of Professor K. MIYABE.

MERRILL¹⁴ has published a second paper describing new plants discovered in the Philippines. It is based mostly on material collected in Bohol during August and September. It includes descriptions of 28 new species, in about as

⁷ RILEY, A. M., Revision of the genus *Calycolpus*. Kew Bull. no. 4. 145-154. 1926.

⁸ CRAIB, W. G., Contributions to the flora of Siam. XVIII. Kew Bull. no. 4. 154-173. 1926.

⁹ KOBUSKI, C. E., A revision of the genus *Priva*. Ann. Mo. Bot. Gard. 13:1-34. 1926.

¹⁰ EPLING, C. C., Studies on South American Labiatae. II. Ann. Mo. Bot. Gard. 13:35-70. 1926.

¹¹ GRENZEBACH, M., A revision of the genus *Bouchea*. Ann. Mo. Bot. Gard. 13:71-100. 1926.

¹² HONDA, M., Revisio Graminum Japoniae. IX. Bot. Mag. Tokyo 40:97-109. 1926.

¹³ ITO, S., and HOMMA, Y., *Miyabella*, a new genus of Synchronitriaceae. Bot. Mag. Tokyo 40:110-113. 1926.

¹⁴ MERRILL, E. D., Additions to our knowledge of the Philippine flora. II. Philippine Jour. Sci. 29:475-496. 1926.

many genera, and also the establishment of 2 new genera of Rubiaceae (*Boholia* and *Sulitia*). The only fern represented is *Schizoloma cordatum*, which is recorded for the first time from the Philippines.

The British Museum is publishing, as a supplement to the *Journal of Botany*, a report of the collection of plants made by JOHN GROSSWEILER in Portuguese West Africa. The 24 pages issued (March-May) include about 90 species, representing 10 families of dicotyledons, of which about 30 are described as new. The largest family is Annonaceae, with 30 species, 12 of which are new; while the Cruciferae are represented by only a single species. The next largest family is Polygalaceae, with 21 species, 19 of them belonging to *Polygala*. The Menispermaceae are also well represented by 17 species, 5 of which are new. The remaining 22 species are distributed among 7 families.

GLEASON,¹⁵ in continuation of his studies of the flora of northern South America, has described 10 new species from British Guiana, representing 8 genera. Two of the genera are published as new, namely *Tetrapodenia* (Malpighiaceae) and *Barnhartia* (Styracaceae).

RYDBERG¹⁶ has published 2 new species from the mountains of West Virginia, *Aconitum vaccarum* and *Heuchera alba*.

SMALL¹⁷ has described a notable new palm, *Sabal Deeringiana*, found growing in the Mississippi delta region. It had been referred to in 1857, in a report of the Mexican Boundary Survey, but "the clue was not followed up." It was recently "rediscovered by mere accident." SMALL¹⁸ has also described a new endemic *Campanula* (*C. Robinsiae*) from Florida.—J.M.C.

Forests of Western Australia.—This part of Australia presents a wide range of climatic conditions within its 900,000 square miles. The rainfall varies from 6 to 70 inches per year, and results in a great variety of forest types which have been well described in recent articles by GARDNER.¹⁹ This author distinguishes sclerophyllous forest in regions with more than 20 inches of rainfall, and among them includes (1) Karri forest, where the precipitation is rather evenly distributed throughout the year. Here the "Karri," *Eucalyptus diversicolor*, is the chief tree, reaching a height of 300 feet, with a lower story of *Agonis*, *Banksia*,

¹⁵ GLEASON, H. A., Studies on the flora of northern South America. IX. Bull. Torr. Bot. Club 53:289-301. 1926.

¹⁶ RYDBERG, P. A., Two new species from the mountains of West Virginia. Torreyia 26:29-33. 1926.

¹⁷ SMALL, J. K., A new palm from the Mississippi delta. Torreyia 26:33-35. 1926.

¹⁸ ———, A new bellflower from Florida. Torreyia 26:35. 1926.

¹⁹ GARDNER, C. A., The hardwood forests. Australian Forestry Jour. 6:185-191. 1923. The forest formations of Western Australia. I: The Karri forest. II: The Jarrah forest. III: The Tuart forest. IV: The Wandoo forest. V: The Salmon gum forest. VI: The Mulga bush. VII: The Tingle-tingle forest. VIII: The Kimberley sclerophyllous woodlands. *ibid.* 6:52-55; 104-108; 199-202; 296-300. 1923. 7:38-45; 120-123; 256-259. 1924. 8:4-6; 72-75. 1925.

Casuarina, *Acacia*, and *Albizzia*, 30-40 feet high; and (2) Jarrah forest, where the rainfall is somewhat more periodic and the "Jarrah," *E. marginata*, grows in rather close stands 75-100 feet high, with an understory of smaller trees. The wood of this Jarrah is hard and durable, being sometimes known as Australian mahogany.

Where the rainfall is 15-20 inches a savanna forest develops, with trees 50 feet high and a grassy undergrowth. Here there are various species of *Eucalyptus*, *Agonis*, and *Acacia*. With less rainfall the vegetation may consist either of poorer savanna or of "mallee scrub," consisting of small scrubby species of *Eucalyptus*, or "Mulga scrub," consisting of *Acacia aneura* and its associates.

The ecological, forestry, and economic relations of the various types are well considered.—GEO. D. FULLER.

Sulphur bacteria.—In a paper which adds little of empirical or theoretical value to our knowledge of the sulphur bacteria, BAAS-BECKING²⁰ describes an ecological community or association of organisms living on the black mud of California lakes. The association is called a sulphuretum, and is limited to alkaline substrates of varying salinity. Nine genera of endothio-bacteria have been found in the region.

The author believes that H_2S is incapable of furnishing the energy for their metabolism, but that it is the HS^- ion which serves as the material for oxidation and energy yield. All observations agree with WINOGRADSKY's classical researches of 40 years ago.

The formation of liquid sulphur globules in the bodies of these bacteria by oxidation of the hydrosulphide ion is interpreted in terms of the glutathione theory of HOPKINS. The reactions tentatively suggested are that oxidized glutathione reacts with HS^- ion to form reduced glutathione and liquid sulphur, S_8 , which appears as droplets within the bodies of the endothio-bacteria. The process of chemosynthesis is thought possibly to involve the interaction of reduced glutathione with H_2CO_3 in such a way as to form H_2O , and the oxidized form of glutathione. In this reaction the H_2CO_3 is reduced to $HCHO$, presumably, which is then condensed to carbohydrate.

The H_2O_2 , on being decomposed by catalase, might furnish oxygen in a form capable of oxidizing the stored sulphur to SO_4 . The suggestions are only tentative, and not very convincing. There seems to be no inherent reason why both ions of the H_2S molecule might not supply hydrogen for the reduction of glutathione.

An error that should have been avoided is the inadvertent one of making glucosamine instead of glutamic acid a part of the glutathione molecule.—C. A. SHULL.

²⁰ BAAS-BECKING, L. G. M., Studies on the sulphur bacteria. Ann. Botany 39:613-650. 1925.

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DISTRIBUTION OF NATIVE PLANTS AND WEEDS ON CERTAIN SOIL TYPES IN EASTERN TEXAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 359

S. R. WARNER

Introduction

No ecologist in traveling through eastern and central Texas could fail to notice how closely plant formations follow certain types of soil in this region. This is strikingly expressed in the formation types of prairie, post oak ridges, and pine land. The entire upland vegetation of the inner coastal plains province is apparently an edaphic complex. So insistent is the nature of the soil in determining the distribution of the vegetation of this region, that one feels that he could assume that all the upland vegetation of this latitude in the inner coastal plains east to Georgia is prairie or forest, depending on the type of soil.

In a study of the correlation between vegetation and certain soil types an area is used on which a soil survey has been made. The soils considered are the most extensive and distinctive in the inner coastal plains province of the southern United States. The writer has attempted to make an analysis of the factors that are dominant in each soil, to define the composition of each edaphic formation, to discover possible stages in plant succession, and to point out the indicator value of certain species and communities. The region of this study may be of general interest because of its age. The prairie soils have never been glaciated, and the sandy soils are leached residues of quartz and silicates, and not mixtures of mineral fragments such

as those usually considered in studies of the upper Mississippi valley and Rocky Mountain regions. Eastern Texas, because of its humidity, affords an excellent opportunity for the observation of other factors than water in determining plant associations.

The literature bearing on the factors determining prairie and forest formations and tension zones is too extensive to be reviewed in the short space of this article. The literature that relates to the use of soil types as a basis for defining plant communities is quite limited. FULLER (10) has pointed out that the forests in the prairie region of Illinois follow certain soil types, and KELLY (11) has considered plants as indicators of soil types in Pennsylvania. A survey of the climatic associations of Texas east of the 98th meridian has recently been made by THARP (17). An ecological survey of the entire state was made earlier by BRAY (1). FOSTER, KRAUSZ, and LEIDIGH (6) made a survey of Texas woodlands. CAMPBELL (2) made a survey of the plant associations in Brazos County, Texas. Several local surveys have been made throughout the area, but the writer has no knowledge of any attempts in Texas to use edaphic conditions as the basis for the formations of plant associations. The classification of plant communities follows the terms defined by NICHOLS (13, 14), the taxonomic nomenclature is based on that of SMALL (16), and dynamic concepts of vegetation are based on the work of COWLES (5) and CLEMENTS (3).

Location and description of soils

In the inner division of the Atlantic and Gulf coastal plains soil province of the southern United States there exist in the uplands two characteristic soil groups differing as to maturity, the leached soils and the calcareous soils. The first group is probably derived from the Piedmont Appalachian and Ozark material, and because of the long weathering of the minerals of these soils they may be considered as old or mature, both as to surface and subsoil. The second group is derived from limestone and chalk, probably transported from western prairies, and consists of unleached soils high in lime and organic content and low in sand. They are very subject to erosion, and it is partly for this reason that they continue their youth, because they are constantly exposing new limy soils from beneath.

The following is a descriptive key, adapted from MARBUT (12), to the soil series of this province which are included in this study:

A. Piedmont Appalachian material.

I. Soils with friable subsoils, drainage well established.

1. Gray soils, subsoil yellow.....Norfolk series
2. Gray soils, subsoil yellow, iron concretions.....Tifton series
3. Gray soils, subsoil light red to reddish yellow.....Ruston series
4. Gray soils, subsoil dull red to normal red.....Orangeburg series

II. Soils with plastic subsoil, drainage well established.

1. Gray soils (reddish gray) subsoil mottled red, yellow.....Susquehanna series

B. Calcareous material.

I. Soils with plastic subsoils, drainage well established.

1. Black soil, subsoil light gray to light brown, highly calcareous.....Houston series

The soils with friable subsoils include four closely related series which differ largely on the basis of color, resulting from the effects of various degrees of weathering on the iron content. The floristic difference of these soils is small and the vegetation will be treated as one association-complex. The Norfolk soil will be considered the type for this group.

The portion chosen for intensive study was the area of the Willis soil survey in Montgomery County, Texas, which is 40 miles north of Houston with a latitude of $30^{\circ} 30'$ and a longitude of $95^{\circ} 30'$. This area is situated just west of the long-leaf pine forest, and is designated by FOSTER (6) as the *Pinus taeda* region, and by THARP (17) as the *Pinus-Quercus* ecotone zone. Reconnaissance studies were made by the writer over much of the area in Texas bounded by longitude meridians 95 and 98, and latitudes 30 and 32. Some study was made of the most easterly extension of the Houston soil in Montgomery County, Alabama, and of the western extension of the Norfolk soils in Bastrop County, Texas. The Alabama prairie occurs on Houston soils about 600 miles east of the central Texas prairies, likewise on Houston soils. The Bastrop area is a disjunct pine forest

80 miles west of other pines to the east, and 120 miles west of the Willis area. Some study was made of the vegetation occurring on the calcareous sands of the eastern coastal strand of Texas. The Willis area contains the dominant soil series of the southern United States in the inner coastal plains province. The most extensive soils of the survey belong to the Norfolk and Houston series. Small areas of Susquehanna, Orangeburg, Lufkin, and Tifton soils occur in this region. The plant associations of these soils were followed into adjoining counties. This study extended over a period of six years, concluding in the year 1925.

Factors

The edaphic formation types seem to be determined by soil texture and aeration, water, and chemical and biotic factors. Other factors of temperature, topography, and transpiration are very important in influencing the communities present within each edaphic formation, but they are not generally applicable in defining the formations of the respective soil types.

SOIL TEXTURE AND AERATION.—The soil structure in itself is an important factor in determining plant distribution. Heavy clays and chalky soils impede root elongation and may influence the development of root hairs. The greatest influence of soil texture is indirectly related to aeration, water capacity, and water movement. Texturally the soil types of this area fall into five classes: (1) the fine sand with fine sand subsoils which have the lowest water capacity and best aeration; (2) the fine sandy loams with friable sand-clay subsoil which permit good aeration, and especially large water capacity in the subsoils; (3) the fine sandy loam with plastic clay subsoils which permit only slow penetration and are poorly aerated in the subsoil; (4) Susquehanna clays with plastic subsoils which possess a high water capacity but puddle badly and lose water rapidly in drought; (5) Houston black clay, with clay surface and subsoil, which crumbles and cracks on drying, and permits ready saturation of the surface but resists percolation and the movement of water and air in the substratum after saturation.

It should be emphasized that when Houston black clay is very wet it is a water-logged soil, and the impervious character of the

chalk and heavy clay of the subsoil so checks the downward movement of water as largely to prevent any movement of oxygen except by diffusion. The poor oxygen supply to the deeper substratum during most of the winter and spring months, when these soils are saturated, is sufficient to act unfavorably on the deep rooted development of many plants. The writer feels that a deficiency of oxygen in the subsoil is one of the operating forces to exclude trees; however, no experimental data were obtained on this point. The difficulty of root penetration in Houston clay is seen in the tendency of the smaller roots to follow cleavage planes, which are less developed in the deeper subsoil. This characteristic itself may be a factor in restricting deep root development.

There is probably a detrimental effect resulting from cracking that may be of ecological significance. The Houston soils, on account of their large water-holding capacity and high content of clay and other colloidal matter, are very subject to checking or cracking. These cracks may reach a depth of more than 3 feet. Lateral roots are normally broken by this shrinkage. The writer observed roots of *Pinus echinata* 0.5 cm. in diameter which were broken in this way. Much greater tensile strength was exhibited by roots of *Toxylon pomiferum* and *Celtis mississippiensis*. Thus it might be said that, since cracking is unfavorable to lateral roots and aeration is unfavorable to deep roots, conditions tend to favor small descending types of root systems. These soils crack before the wilting coefficient is reached. Blocks of soil are sufficiently large to accommodate the root system of most grasses.

Table I summarizes the mechanical analyses of twenty-seven soil samples of eastern and central Texas counties. This table and the foregoing descriptions of the soil types give the best picture of the texture and probable aeration of the respective soils.

WATER.—The soils of the regions under consideration are normally supplied with an adequate rainfall. The averages are: Willis, Texas, 45 inches; Bastrop, Texas, 35 inches; and Montgomery County, Alabama, 51 inches. The rainfall evaporation ratio of 90 per cent for Alabama and Willis, Texas, is well above the 80 per cent emphasized by SAMPSON (15) as the climatic limit of prairies. The rainfall in the year from June 1924 to July 1925 was very light:

Willis 13.35 inches; Bastrop 13.39 inches; and Montgomery 37.5 inches (24 inches of this fell during December, January, February, and March). This is evidence that at times there may be extreme departure from the average when the water reserve of the soil becomes an important factor in the life of vegetation. Determinations of the amount of water in the respective soils at the end of drought periods are shown in table II.

TABLE I
SUMMARY OF AVAILABLE MECHANICAL ANALYSES OF CERTAIN
SOILS IN EASTERN TEXAS*

SOIL	NO. OF SAMPLES	COARSE AND MEDIUM SAND		FINE SAND		VERY FINE SAND		TOTAL SURFACE SAND		TOTAL SUBSOIL SAND		SILT		CLAY		TOTAL SURFACE SILT AND CLAY		TOTAL SILT AND CLAY SUBSOIL	
		Soil	Subsoil	Soil	Subsoil	Soil	Subsoil					Soil	Subsoil	Soil	Subsoil				
Norfolk fine sand.....	5	4.7	5.38	62.4	62.6	18.1	16.7	85.2	84.6	12.2	11.5	2.8	3.8	14.8	15.4				
Norfolk fine sandy loam...	5	0.65	0.6	31.5	31.7	39.3	26.1	71.4	57.7	23.1	19.9	4.96	21.7	28.1	41.6				
Orangeburg fine sandy loam.....	2	2.6	2.2	35.4	21.2	24.2	22.4	62.2	45.8	31.2	29.2	6.4	24.	37.6	53.2				
Susquehanna fine sandy loam.....	6	1.45	.85	35.6	27.2	27.3	16.9	64.4	44.9	24.9	17.9	8.2	37.0	33.1	54.9				
Susquehanna clay.....	3	2.6	1.2	20.5	9.1	26.8	13.3	49.9	23.6	38.2	25.5	12.4	49.8	50.6	76.3				
Houston clay.....	6	1.7	1.9	7.9	5.8	11.0	8.0	20.6	15.7	49.1	50.2	30.0	33.2	79.1	83.4				

* Figures compiled from numerous reports of soil surveys of Texas counties by the United States Bureau of Soils.

From these figures it is evident that the amount of available water at a depth of 24 inches in the uncultivated Houston soil, the prairie type, is as great as it is in the other uncultivated soils at this depth, and none of these soils possess available water at a depth of 12 inches. These figures also indicate that cultivated Houston black clay has a greater water retaining capacity than the Norfolk and Susquehanna soils. This fact is supported by observations of crops under cultivation on the soil types during prolonged dry periods. Especially is this noticeable when comparison is made between Houston soils and the tight silty post oak soils. The greater water retaining capacity characteristic of the Houston

soils is associated with the high content and greater depth of organic matter, the texture, and the flocculent character of the soil. The presence of lime in these soils aids in the retention of soil water by causing the soil surface to crumble on drying, thus forming a mulch. The severe cracking or checking of this soil in drought periods is an important influence in water loss, but its importance is usually over estimated. The cracking does not indicate that the soil is necessarily drier but that it shrinks on drying. It is because of these characteristics that summer rains are readily absorbed with little or no run-off. The reaction of vegetation to drought in prairies of the

TABLE II
WATER IN SOIL IN AUGUST AT END OF 60-DAY DROUGHT PERIOD

	NORFOLK FINE SAND		NORFOLK FINE SANDY LOAM		SUSQUEHANNA RED CLAY		HOUSTON BLACK CLAY	
	Depth of sample (percentage)							
	12 in.	24 in.	12 in.	24 in.	12 in.	24 in.	12 in.	24 in.
Wilting point*.....	1-2	1-2	3.1	3.1	10.3	10.3	16.1	16.1
<i>Pasture sod</i>								
Total water.....	1.8	2.1	2.8	5.5	9.5	13.6	14.5	20.2
Available water.....	0.0	Trace	0.0	2.4	0.0	3.3	0.0	4.1
<i>Cultivated soil</i>								
Total water.....	3.3	4.2	6.0	11.4	1.1	21.0	30.7	34.8
Available water.....	1.3	2.2	2.9	8.3	2.0	11.0	14.6	18.7

* Wilting point determined on basis of hygroscopic moisture.

Houston clay seems to depend more on the character and extent of root systems of the plant and movement of water in the soils than on their water content. The tree growth forms apparently find the Houston soils more xerophytic than the fine sandy loams; this is true at least in the late summer and fall months during a prolonged drought. The other soils, which show xerophytism, usually manifest this condition early in the season on herbaceous growth forms. The four most xerophytic habitats are the ridges of deep Norfolk sand, the eroded slopes of Susquehanna clay, the shallow chalky phases of the Houston soils, and the silty clay transitional soils (Lufkin type) between true prairie and true woodland.

The following observations may best express the reaction of the vegetation to the water factor in the drought period of 1924-1925.

In the xerophytic sandy situations there was little dying of larger pines, but there was a high fatality among pines under five years of age and in upland hardwoods, as *Quercus digitata*, *Q. marylandica*, and *Hicoria glabra*. In the margins of the Houston soil there was considerable dying of pines of all ages, also of *Quercus digitata*, *Q. marylandica*, *Hicoria villosa*, *Crataegus viridis*, and *C. spathulata*, but little effect was shown on *Q. minor*, *Celtis mississippiensis*, *Toxylon pomiferum*, and *Ulmus crassifolia*. The apparent reason for the greater drought tolerance of pines in Norfolk sand than in Houston soils is because the loose texture of Norfolk soil is favorable to the inherent tendency of *Pinus taeda* and *P. echinata* to form deep extensive root systems. In the transitional zone between prairie and forest there occurred the greatest fatality of tree growth forms. This was especially noticeable among older trees. At many places in this zone as high as 20 per cent of the pines were lost. Almost as great a loss occurred in *Q. digitata*. It is interesting to note that those species which showed the greatest drought tolerance were the ones showing the greatest defoliation, namely, *Q. minor*, *Celtis mississippiensis*, *Ulmus crassifolia*, *Toxylon pomiferum*, *Populus deltoides*, and *Morus rubra*.

Topography and transpiration are important factors in determining the water relations of a community, but since the edaphic formations of this region occur within a few yards of each other and occupy equally exposed situations, it seems unjustifiable to consider either of them of importance in determining the edaphic formations under consideration. All prairies of this area occur on upland Houston soil and are the result of characteristics possessed by this soil. There is little apparent relation between their occurrence and the direction of the slope or the position of exposure. Small prairies sometimes exist on northern slopes and are surrounded by virgin forest. Some of these show only a few yards of forest invasion in a period of many centuries.

That water is an important factor is shown by the xerophytic, semixerophytic, mesophytic, and hydrophytic divisions of edaphic formations. Since these parallel communities are not similar in leached and unleached soils, water apparently is not the controlling factor in determining the difference in the structure of edaphic

formations under consideration. This is most strikingly demonstrated by the dissimilarity in the societies of winter annuals and weeds on cultivated soils which are little affected by the water differences. The conditions of the leached upland ridges apparently are more xerophytic than those of the exposed prairies. The vegetation of the former shows a higher fatality than that of the latter; therefore neither transpiration nor soil water explains adequately the difference in the structure of these communities.

CHEMICAL FACTORS.—The leached and unleached soils which support unlike plant formations show marked chemical differences. These chemical differences have been investigated from the standpoint of soil reaction with regard to the hydrogen-ion concentration and amount of titratable acid, and from the standpoint of composition of the soil, with especial consideration given its calcium carbonate content. The colorimetric method recommended by WHERRY (21) has been followed in determining the P_H , and the results are summarized in table III. The majority of these determinations were made during the drought period of 1924–1925. The P_H of the leached soils is seen to be characteristically acid; that of the unleached Houston soil is alkaline. The acidity seems to be of two origins. One is associated with surface organic matter, as in woods where it is greatest near the surface and decreases with the depth of the soil in soils containing little clay. The other type of acidity is associated with the mineral constituents of the soil. It increases proportionately with the clay content to a depth of about 10 inches; below this the substratum becomes less acid. This indicates that the acidity is probably related to the accumulation of weathered aluminum and iron silicates at this depth. In the soils low in organic matter, as in sterile old fields, the deep Norfolk fine sand was nearly neutral, due to the almost negative action of the sand; the fine sandy loams were more acid; and the Susquehanna soils highly acid, due to the influence of increased mineral acids from the content of clay. The poorest growth of vegetation occurs upon these leached and acid slopes. The slopes of the Norfolk soil show a greater acidity than its more level areas, due to the effects of leaching; the slopes of the Houston clay are more alkaline than its level areas, due to the loss of surface soil by erosion.

The difference in the influence of carbon dioxide on roots in acid and in alkaline soils may have some influence on plant distribution.

In leached soils carbon dioxide is always present as carbonic acid acting upon the roots and the soil. This is shown in the acidity of spring water in these areas. Examination of ten such springs gave

TABLE III
SOIL REACTION EXPRESSED IN POTENTIAL HYDROGEN

FORMATION TYPE	SURFACE SOIL				SUBSOIL	
	Low	High	Average	Character	10 in.	3 ft.
<i>Willis area</i>						
Houston black clay						
Xerophytic.....	7.5	9.0	8.5	8.5	8.5	8.5
Semixerophytic.....	7.0	8.5	8.0	8.0	8.5	8.5
Mesophytic.....	6.0	8.0	7.4	7.2	7.5	8.0
Hydro-mesophytic.....	7.0	8.0	7.5	7.5	7.5
Susquehanna reddish clay						
Xerophytic.....	4.8	6.5	5.4	5.5	5.2	5.5
Norfolk fine sandy loam						
Semixerophytic woods.....	5.2	7	5.8	5.8	5.5	5.8
Semixerophytic cultivated...	5.4	7.2	6.2	6.2	5.8	5.8
Semixerophytic sterile old fields.....	5.0	6.5	5.6	5.5	5.2	5.5
Mesophytic.....	5.2	7.2	5.8	5.8	6.0	6.0
Hydro-mesophytic seepy slopes	4.8	6.0	5.2	5.0	5.2
Norfolk deep fine sand						
Xerophytic woods.....	4.8	7.0	6.1	6.0	6.4	6.5
Xerophytic new ground.....	5.8	7.5	6.6	6.8	6.5	6.5
Xerophytic sterile old fields..	6.0	7.0	6.5	6.5	6.2	6.5
<i>Bastrop area</i>						
Susquehanna gravelly loam....	4.5	7.0	5.5	5.2	5.0	5.0
Norfolk fine sand.....	5.2	7.5	6.3	6.4	5.7	5.8
<i>Other areas</i>						
Beach strand (marly sands)....	7.5	9.0	8.5	8.5	8.5
Orangeburg fine sandy loam....	5.4	6.8	6.2	6.0	5.8	6.5
Peaty mucks.....	4.2	4.8	4.5	4.5	4.5
Alluvial soils.....	5.5	8.5	7.2	7.0	7.4	7.0

an average acidity of P_H 5.4, which could be destroyed by boiling. In Houston soils the acid action of excreted carbon dioxide is quickly lost by its combination with the calcium carbonate to form a bicarbonate. If carbon dioxide can have any influence on plant distribution, therefore, it should have this effect in leached and limy soils where it occurs in different conditions.

The Norfolk and Susquehanna soils were tested by the VETICH (18) method for specific acidity. Five tests were made for each soil,

and every analysis of the uplands of these two soils showed an absorption of the base by the soil. The acidity in parts per million of the Susquehanna soil ranged from 400 to 2100; that of the Norfolk sand ranged from 50 to 500; and that of the Norfolk fine sandy loam ranged from 0 to 700.

The Houston soils are rich in lime. Ninety-nine samples out of every hundred taken at random give an active effervescence when treated with hydrochloric acid. Quantitative determinations from three typical fields of this soil type gave the following percentages of

TABLE IV

CHEMICAL CONSTITUENTS OF CERTAIN SOIL TYPES IN EASTERN TEXAS

SOILS	NO. OF SAMPLES	PHOSPHORIC ACID	NITROGEN	POTASH	LIME	MAGNESIA	IGNITION LOSS	NO. SAMPLES SHOWING ACIDITY	AVERAGE ACIDITY OF ACID SAMPLES
Norfolk sand and fine sand..	9	0.02	0.04	0.15	0.13	0.11	1.99	2	250
Norfolk fine sandy loam.....	7	0.02	0.03	0.09	0.12	0.07	1.25	7	309
Orangeburg sand.....	1	0.02	.02	.15	.05	.09	0.17	1	200
Orangeburg fine sand.....	1	0.01	.02	.07	.14	.14	0.94	1	300
Orangeburg fine sandy loam.	2	0.02	.02	.10	.10	.08	1.26	2	325
Susquehanna fine sand.....	2	0.02	.02	.10	.05	.07	1.08	2	450
Susquehanna fine sandy loam.	5	0.02	.03	.16	.09	.10	1.80	3	660
Ruston fine sandy loam.....	2	0.02	.03	.10	0.14	0.07	1.66	1	600
Houston black clay.....	5	0.18	.12	.49	7.6	1.15	8.25	0	0
Houston black clay.....	5	0.06	.10	.55	.84	.78	5.27	1	50
Susquehanna clay.....	1	0.07	0.13	0.44	0.26	0.49	7.68	1	330

CaO: surface soil 4.28, 4.06, and 5.02; subsoil 10.24, 7.63, and 12.48. Similar analyses of the more calcareous spots gave a CaO content of 7.75 per cent for the surface and 13.63 per cent for the subsoil.

These determinations show high base content in Houston soils, and a general deficiency of bases in the Susquehanna and Norfolk soils. That similar conditions exist for these respective soils throughout eastern Texas is shown in table IV, which is a summary of analyses of these soils made by FRAPS (7, 8, 9). The close correlation between soils of high basic content and the occurrence of prairie species, which exist in edaphic formations of this region, holds true for the climatic prairies to the west. A similar correlation between edaphic formations of leached soils in this region exists for

the climatic forest to the east. In a compilation of averages from COFFEY (4), the prairie states of Nebraska, Kansas, and Iowa showed an average analysis of potash 0.45, phosphoric acid 0.22, lime 0.80, and magnesia 0.32. The southeastern timbered states of North Carolina, South Carolina, and Georgia show potash 0.12, phosphoric acid 0.08, lime 0.13, and magnesia 0.11. These figures indicate that the chemical nature of the soil may be as important a factor in determining the vegetation of climatic formations as in determining edaphic formations.

BIOTIC FACTORS.—That microorganisms may play an important rôle in developing soil characteristics and plant associations is quite

TABLE V
NUMBER OF MICROORGANISMS PER GRAM IN PASTURE SOIL FOR
MEDIA AND DETERMINATIONS*

	NORFOLK FINE SANDY LOAM PH 5.8	SUSQUEHANNA RED CLAY PH 5.2	HOUSTON BLACK CLAY PH 8
Bacteria			
Media PH 7	8,600,000	3,400,000	14,800,000
Media PH 5.4	7,700,000	3,700,000	9,300,000
Actinomycetes	24 per cent	10 per cent	37 per cent
Bacteria	76 per cent	90 per cent	63 per cent
Soil fungi	63,000	44,300	14,400

* WAKSMAN and FRED'S (19) methods.

apparent, but this factor is difficult of analysis. The development of black humus and black soils in the presence of lime and the development of gray soils in a deficiency of lime are probably associated with the activity of soil organisms. WAKSMAN (20) concludes that the greater humus in black soil is due to greater carbon residue from bacterial action, since bacteria use carbon less effectively than fungi. The two groups of soils contrasted in this study are respectively gray and black; therefore, some analysis of this factor becomes necessary (table V).

The observations made from the study of microorganisms may be summarized as follows: (1) Bacteria are more numerous in unleached black soils and fungi are more abundant in gray leached soils. (2) Many bacteria of the black soils which will develop in neutral or alkaline media will not develop in this same medium which has had its acidity increased to a point comparable with the

acidity existing in gray soils. (3) The fungi of the two types of soil show quantitative and qualitative differences, the latter being perhaps of more importance; these the writer hopes to treat in a later paper. (4) Since it is true that the distribution of many microorganisms is dependent on certain chemical conditions, it is reasonable to assume that many seed plants are influenced by such conditions. This relation may have a direct influence in the rôle of parasitism, symbiosis, and the favorable toxic products of microorganisms.

Many animals show an ecological distribution which correlates with two edaphic formations. This is most noticeably true in case of snails and certain insects, particularly grasshoppers. Earthworms are the most interesting because of their effect on soil conditions. They occur mostly in mesophytic conditions, and show a decided preference for the neutral to slightly alkaline Houston and alluvial soils. Here they are larger and more numerous than in the moist sour Susquehanna clay or Norfolk sandy soils. Their presence probably has some effect on these mesophytic associations.

Grazing affects all the formation types, and often obscures the evidence of dominant forms. Overgrazing is usual during certain seasons. The species of *Andropogon*, the dominant native grasses, are suppressed almost completely in such areas. *Capriola dactylon* takes almost complete possession of the better soils. *Axonopus compressus* forms an almost continuous mat over the less productive fine sandy loams and moist places in the Susquehanna clay. The *Aristata* grasses are the most frequent on sterile xerophytic habitats. In sands they are accompanied by several *Panicum* grasses, and on limy clays by *Buchloe dactyloides* westward of the Willis area. Grazing favors pine forest perpetuation, but prairie continuance is not favored by grazing. This latter has been emphasized by BRAY (1).

Classification and description of plant communities

The most natural and consistent division of this climatic region is on the basis of soil maturity, the edaphic formation type of the leached potentially acid soils, the edaphic formation type of the unleached alkaline soils, and the edaphic formation complex of the mixed soils, circumneutral in their reactions.

A. EDAPHIC FORMATION TYPE OF UPLAND LEACHED (OLD) SOILS

1. XEROPHYTIC FORMATION, TYPE OF UPLAND RIDGES

Quercus minor and *Q. marylandica* are the most constantly occurring tree forms in this formation type, and generally become subdominant to *Pinus echinata* east of the 96th meridian. These dominant species do not show a high coefficient of fidelity to this formation.

(a) ASSOCIATION TYPE OF LOOSE, CONSTANTLY AERATED SOILS.—The following plants show considerable fidelity to this type. Trees and shrubs: *Pinus echinata*, *Quercus brevifolia*, *Q. marylandica*, *Castanea pumila*, *Prunus milis*, *Schmalzia aromatica*, *Ascyrum hypericoides*, *Ceanothus americanus*, *Vitis Linescomii*, and *Croton argyranthemus*. Grasses: *Andropogon scoparius*, *Stipa avenacea*, *Aristida intermedia*, and *Cenchrus tribuloides*. Herbaceous perennials: *Morongia angustata*, *Astragalus obcordatus*, *Lepedeza hirta*, *Cnidoscolus texanus*, *Asclepias tuberosa*, *A. amplexicaulis*, *Lithospermum Gmelini*, *Physalis intermedia*, *Laciniaria elegans*, *Berlandiera dealbata*, *Froelichia campestris*, and *Chrysopsis graminifolia*. Annuals: *Richardia scabra*, *Linaria texana*, *Heterotheca subaxillaris*, and *Isopappus divaricatus*.

(b) ASSOCIATION TYPE OF POORLY AERATED SOILS WITH PLASTIC CLAY SUBSOILS.—The following plants reach their greatest frequency in this association, although none of them are restricted to it. Trees and shrubs: *Quercus minor*, *Hicoria glabra*, *Diospyros virginiana*; also *Panicum anceps*, *Aristida purpurascens*, *Lepedeza procumbens*, *Meibomia obtusa*, *Stylosanthes biflora*, *Plantago aristata*, and *Chaetopappa asteriodes*. This association is poorly defined and limited in its occurrence, appearing mainly on outcrops of Susquehanna clay. It is a very dry soil in drought periods due to puddling and evaporation, and is usually an acid soil.

2. THE SEMIXEROPHYTIC FORMATION OF ORDINARY UPLANDS

This type embraces all the fine sandy loams of the Norfolk, Tifton, Ruston, Orangeburg, and Susquehanna soil series, occurring upon the ordinary uplands east of the 96th meridian. It constitutes about 90 per cent of the forest in this region, and is the present forest

climax of this character of soil. No attempt will be made to split this formation into its poorly defined associations. Pines are the dominant species, except in a few limited areas where the better phases of the Orangeburg and Susquehanna approach the Greenville series in a higher content of bases. *Pinus echinata* is more xerophytic and more northern in its frequency distribution than *P. taeda*. Where subsoils are rich in ferruginous compounds and usually high in acidity, the pine that is most likely to occur is *P. echinata*. Trees which occur occasionally are *Quercus digitata*, *Liquidambar styraciflua*, and *Ulmus alata*. *Callicarpa americana* is dominant throughout the forests of this type. *Ilex vomitoria* is the most frequent shrub in sunny situations in the southern part of this area. Other plants that reach their greatest frequency in this formation type are *Andropogon virginicus*, *Festuca octoflora*, *Panicum lanuginosum*, *Tridens flava*, *Trifolium carolinianum*, *Cerastium viscosum*, *Syntherisma sanguinale*, *Mollugo verticillata*, *Amaranthus spinosus*, *Hedeoma hispida*, *Rumex hastatulus*, *Salvia lyrata*, *Plantago virginica*, *Viola septemloba*, *Vernonia texana*, *Eupatorium compositifolium*, *Rudbeckia hirta*, and *Diodia teres*.

3. MESOPHYTIC FORMATION TYPE OF LOWER SLOPES AND FLATWOODS

This association complex is protected from high transpiration by topographical and vegetational features. The soil water is usually abundant, and the water table is at a shallow depth. *Pinus taeda* is the dominant tree, being at least ten times as frequent as *P. echinata*. *Liquidambar styraciflua* is next in importance in its dominance. *Quercus alba* is usually present but is dominant in very limited situations. *Magnolia foetida* and *Ilex opaca* occur mainly in the proximity of stream courses. *Myrica cerifera* is the dominant shrub, especially where the soil structure is loose and the water table shallow; *Callicarpa* prefers well drained soils. Other shrubs are *Viburnum molle*, *Rubus argutus*, and *Gelsemium sempervirens*. Herbaceous plants are *Uniola longifolia*, *Axonopus compressus*, *Eupatorium coelestinum*, *E. semiserotinum*, *E. rotundifolium*, *Aster vimineus*, *Solidago celtifolia*, *Elephantopus carolinianus*, *Lespedeza stricta*, and *Serinea oppositifolia*.

4. HYDRO-MESOPHYTIC TYPE OF WET SOUR SOILS

This formation includes the associations of wet sour habitats such as seepy slopes and sandy swamps, and does not include the ordinary lowlands and stream margins. Such soils are usually neutral or slightly alkaline in their reaction, and have been included in the type of the alluvial mixed soils. Some plants in the following list were not observed in the Willis area but occurred in sandy swamps nearby. Trees and shrubs: *Magnolia virginiana*, *Nyssa sylvatica*, *Acer rubrum*, *Myrica cerifera*, *Rhus vernix*, *Vaccinium corymbosum*, *V. stamineum*, *V. virgatum* var. *tenellum*, *Smilax laurifolia*, *Azalea viscosa*, and *Ascyrum hypericoides*. Herbaceous plants: *Xyris difformis*, *Drosera brevifolia*, *Sarracenia flava*, *Eriocaulon decangulare*, *Juncus validus*, *J. scirpoides*, *Panicum rostratum*, *Ibidium cernuum*, *Phlomis capillaceum*, *Ludwigia alternifolia*, *L. glandulosa*, *Lobelia puberula*, *Eupatorium rotundifolium*, *Pluchea foetida*, *Osmunda regalis*, *O. cinnamomea*, and *Sphagnum* sp.

B. EDAPHIC FORMATION TYPE OF UPLAND
UNLEACHED SOILS1. XEROPHYTIC FORMATION TYPE OF SHALLOW, ERODED,
LIMY SOILS

This is the driest, best aerated, most calcareous, least organic and least productive soil of the Houston series. The most characteristic ecological forms are the herbaceous perennials *Andropogon saccharoides* var. *laguroides*, *Melilotus alba*, *Petalostemon multiflorus*, *P. purpureus*, *Astragalus crassicaepus*, *Verbena bipinnatifida*, *Houstonia angustifolia*, *Grindelia lanceolata*, and *Rudbeckia missouriensis*. These show a high constancy and fidelity to this habitat. Other species showing a preference for this habitat are *Ceanothus ovatus*, *Aristida oligantha*, and *Heliotropium tenellum*. One of the most gorgeous wild flowers in America, the Texas bluebell (*Eustoma Russellianum*), occurs in profusion on this and the following type. Attempts to grow it on any but a limy soil have been unsuccessful.

2. SEMIXEROPHYTIC FORMATION TYPE OF
ORDINARY UPLANDS

This association complex is typically prairie, and occupies the most extensive area of this soil type. The soil is characteristically

black, deep, and well supplied with plant nutrients. The pioneer dominant is *Sorghum halepense*. The climax dominants are arranged according to their rank. *Andropogon saccharoides* var. *laguroides*, *Sporobolus Drummondii*, *Stipa leucotrica*, and *Andropogon furcatus*. Trees and shrubs occur sparingly in certain favorable situations: *Ulmus crassifolia*, *Celtis mississippiensis*, *Toxylon pomiferum*, *Gleditsia triacanthos*, *Quercus texana*, *Prosopis glandulosa*, *Sida asperifolia*, and *Crataegus viridis*. Perennials: *Acuan illinoensis*, *A. leptoloba*, *Hartmannia speciosa* (white form), *Vicia texana*, *Physalis longifolia*, *Silphium asperum*, *Helianthus Maximilianii*, and *Solidago altissima*. Annuals: *Panicum capillare*, *P. fasciculatum*, *Amphicarys dracunculoides*, *Bifora americana*, *Plantago rhodosperma*, *Draba cuneifolia*, *Monarda dispersa*, *Crotón monanthogynus*, *Centaurea americana*, and *Lindheimera texana*.

3. MESOPHYTIC FORMATION TYPE OF LOWER SLOPES AND MORE LEVEL SOILS

This type has a lower alkalinity and transpiration rate than the preceding. Trees tend to dominate this association. All of the trees mentioned in the semixerophytic type occur here. Other trees and shrubs are *Fraxinus americana*, *Morus rubra*, *Ulmus americana*, *Cercis canadensis*, *Ilex decidua*, *Smilax Bona-nox*. Grasses: *Andropogon furcatus*, *A. glomeratus*, *Tripsacum dactyloides*, *Sorghum nutans*. Herbaceous perennials: *Hartmannia speciosa* (pink form), *Dolicholus minimus*, *Neptunia lutea*, *Mesadenia tuberosa*, *Aster salicifolius*, and *Carduus austrinus*. Annuals: *Dichrophyllum bicolor*, *Hordeum pusillum*, *Leptochloa mucronata*, *Chaetochloa lutescens*, *Tropocarpus aethusae*, *Chamaesyce nutans*, *Astragalus Nuttalliana*, *A. reflexus*, *Dracopsis amplexicaulis*, *Senecio lobatus*, and *Sitilias multicaulis*.

4. HYDRO-MESOPHYTIC AND HYDROPHYTIC FORMATION-COMPLEX OF ALKALINE DEPRESSIONS AND LOWLANDS

Trees and shrubs: *Fraxinus lanceolata*, *Ulmus americana*, *Hicoria aquatica*, *H. pecan*, *Populus deltoides*, *Planera aquatica*, *Gleditsia aquatica*, *Ampelopsis cordata*, and *Vitis cinerea*. Herbaceous plants: *Uniola latifolia*, *Elymus virginicus*, *Lythrum ovalifolium*, *Meibomia paniculata*, *Koellia flexuosa*, *Iva ciliata*, *Ambrosia trifida*,

Helianthus Maximilianii, *Helianthus grosseserratus*, and *Aster exilis*. The aquatics are *Chara*, *Potamogeton*, and *Typha*.

C. EDAPHIC FORMATION TYPE OF MIXED SOILS, USUALLY NEUTRAL IN REACTION

1. TRANSITIONAL ZONE BETWEEN LEACHED AND UNLEACHED UPLAND SOILS

This is the tension zone between prairie and forest. These soils are much the same as those of the Lufkin series. They normally possess silty fine sandy loam surface soil and a plastic clay subsoil. This type ranges from mesophytic to xerophytic conditions, dependent upon the topography and drainage. *Quercus minor* is the dominant tree. *Crataegus spathulata*, *Celtis mississippiensis*, *Prunus americana*, and *Pinus taeda* are the dominant species. Mesophytic situations are particularly favorable to the development of *Pinus taeda*. *P. echinata* is infrequent in this zone. Other plants showing a preference for this zone are *Chamaecrista fasciculata*, *Sabbatia campestris*, *Panicum virgatum*, *Laciniaria pycnostachya*, *Gaura longiflora*, *Symphoricarpus symphoricarpus*, *Ruellia ciliosa*, *Ptilimnium Nuttallii*, and *Erigeron tenuis*.

2. ASSOCIATIONAL COMPLEX OF ALLUVIAL SOILS

The soils of this association are usually slightly alkaline and well supplied with nutrients. This perhaps explains why the associations of the Houston soils are better represented here than on the leached upland soils. This formation complex also contains a large number of plants of the upland sands. It is here that the mesophytic types of the two edaphic formations merge into one complex. The hardwoods are the dominant trees. Lianas reach their best expression in this habitat.

Plant communities of special indicator significance

1. DISJUNCT PRAIRIES OF MONTGOMERY COUNTY, ALABAMA.—This is a natural upland prairie on Houston black clay. It presents much the same ecological and floristic aspect as is observed on this soil type in Texas. Estimates on an undisturbed area indicate that 75 per cent of the plant cover is occupied by species common to the

Texas prairies, as *Andropogon saccharoides*, *Sporobolus Drummondii*, *Hartmannia speciosa*, *Monarda dispersa*, *Croton monanthogynus*, *Acuan illinoensis*, *Petalostemon purpureus*, *Houstonia angustifolia*, *Heliotropium tenellum*, and *Polytaenia Nuttallii*. Common weeds in disturbed areas are *Rudbeckia amplexicaulis*, *Iva ciliata*, *Ambrosia trifida*, *Aster exilis*, *Trepocarpus aethusae*, *Sida cordifolia*, and *Sorghum halepense*. These species are normally absent from sandy areas nearby. This prairie formation on upland calcareous soils in the midst of the eastern climatic forest is an indication that the vegetation expresses the youth or maturity of the soil.

2. DISJUNCT PINE FOREST OF BASTROP COUNTY, TEXAS.—This area is 80 miles west of other pine forest and in the tall grass prairie region of the state. *Pinus taeda* forms a typical pine forest on an area of 40 square miles of Norfolk fine sand and a small amount of loose Susquehanna gravelly loam. To recount the species common to this and to the pine forest of the Willis area would make too long a list. Mention may be made of some of the more interesting members: *Sida florida*, *Vitis Lenscomii*, *Myrica cerifera*, *Drosera brevifolia*, *Laciniaria elegans*, *Eupatorium coelestinum*, *Hypoxis hirsuta*, *Ilex vomitoria*, *Cracca virginiana*, and *Isopappus divaricatus*. The most notable for its absence is *Pinus echinata*. The rather xerophytic habitat suggests that it rather than *P. taeda* would be expected here. Two possible causes for its absence are suggested: (1) that *P. echinata* extended its range southward in Texas since this area was cut off from the eastern forest; (2) that *P. echinata* is less tolerant of high temperatures than *P. taeda*. This area occurs in the latitude of the southern limit of *P. echinata*. This disjunct area is an example of a pine forest being retained west of its climatic range by edaphic conditions.

3. CALCAREOUS SANDS OF COASTAL STRAND OF EASTERN TEXAS.—Many species common to leached sour sands are lacking in these soils, and many species common to calcareous Houston clay occur on these calcareous sands, although they are rare on leached sands contiguous to the prairies. The following plants, frequent upon the calcareous soils of the prairie, occur here: *Acuan illinoensis*, *Dichrophyllum bicolor*, *Chamaesyce nutans*, *Croton monanthogynus*, *Helianthus Maximilianii*, *Gaura parviflora*, *Iva ciliata*, *Ambrosia trifida*,

Grindelia lanceolata, *Houstonia angustifolia*, *Dolicholus minimus*, *Scutellaria Drummondii*, *Gleditsia triacanthos*, and an occasional *Quercus virginiana*. The preference of these species for unleached soils seems to be independent of soil texture and aeration. A number of other plants show a preference for sand that is not correlated with a leached condition, as *Heterotheca subaxillaris*, *Daucus pusillus*, *Monarda punctata*, and *Erythrina herbacea*. Some show a preference for unleached sand, as *Gaillardia pulchella* and *Argemone alba*.

4. BIG THICKET OF TEXAS.—A mesophytic climax forest type much like the Tennessee forest type joins the *Pinus taeda* forest of the Willis area on the east, and is known locally as the Big Thicket because of the luxuriance of its undergrowth. Its area covers about 1000 square miles, and its shape is that of a delta, with the apex inland near Livingston and its base extending almost to the coastal prairies to the south. On the eastern boundary it is joined by the *P. palustris* forest of the coastal flatwoods. Its topography is much the same as that of the adjoining region, except that it is traversed by the Trinity River and its floodplains, and is more broken by stream courses making it less subject to fires. The soils are fine sandy loams known locally as hammock lands, and are circumneutral in their reaction. The dominant species are *Quercus alba*, *Q. phellos*, *Fagus americana*, *Acer saccharum*, *Magnolia foetida*, and *P. taeda*. It seems improbable that this climax vegetation and the accompanying circumneutral condition of the soils would become general throughout eastern Texas on leached uplands as a result of increased mesophytism. This formation is really south of its climatic range, and owes its preservation to local influences that protect it against the ordinary extremes of climate and to a soil which is probably younger and more fertile than other sandy loam soils of the adjacent areas.

5. PLANT COMMUNITIES AS INDICATORS OF SOIL RELATIONSHIPS.—A good idea of the edaphic formation relationships and distinctions was obtained by determining the number of plants that were frequent on one soil and infrequent on an associated soil. The following is a summary of the species having preference for certain soil types, determined by their percentage of frequency. Of the 178 species frequent on Norfolk sand, 118 seldom occur on Houston black clay,

20 seldom occur on red clay, and 11 seldom occur on Norfolk fine sandy loam. There are 161 species frequent on Houston black clay. Of this number, 101 seldom occur on sand. On red clay there are 163 frequent species. Of this number, 103 seldom occur on Houston black clay and 5 seldom occur on Norfolk sand or Norfolk fine sandy loam. These figures show the close correlation between the vegetation in soil types of low basicity, and they indicate that the plants of Norfolk sand and Norfolk fine sandy loam and Susquehanna clay should be included in one plant formation. Except for a small number of indicator species, differences in vegetation of these soils are largely differences of valence of species.

TABLE VI
INDICATOR SIGNIFICANCE OF ANNUALS IN NORFOLK
AND HOUSTON SOILS

HABITAT	ALL ANNUALS	WINTER ANNUALS	SUMMER ANNUALS
Plants showing a strong preference for			
Sandy soils.....	50	32	28
Black clay soils.....	55	31	24
Plants common to both.....	72	40	32
Total.....	177	103	84

6. ANNUALS AS INDICATORS.—Annuals are important indicators, especially in cultivated ground, as shown in table VI. Under the winter annuals are included all those species that occur from seeds germinated by fall rains, and that mature their seed before the middle of June. Vegetation during this period seldom suffers from lack of moisture. That the percentage of difference for the winter annuals of the Houston black clay and of the sandy soil types should be little greater than that of summer annuals is an argument against considering water as the dominant factor in accounting for the differences in the vegetation of these soils. The most striking indicators of sufficient calcium carbonate in a soil are observed in two summer annuals, *Dichrophyllum bicolor* and *Amphiachyris dracunculoides*. These are so frequent in late summer and autumn that non-acid soils may be excellently mapped by their distribution.

Succession

Succession in this region is associated with the slowly operating processes, leaching and leveling. The associations are so nearly stable that any prediction of change must be largely speculative. The two most extensive and best developed associations are the pine forest and the prairie, but these are apparently the least stable ones, and there is evidence in places of their losing ground. These also are the most aggressive pioneering associations. Their quick invasion following catastrophes is sufficient explanation of their occupation of many situations.

The recent more rapid invasion of prairies by members of other associations is a result of overgrazing which permits the entrance of certain woody species, but the slower and more permanent invasion occurs under natural conditions as a result of leaching, change of texture, and stabilization of the soil. These conditions bring in the xerophytic low oak associations in dry situations, and the tall broad-leaf hardwoods and *Pinus taeda* in the less dry situations. Thus it would seem that the prairie areas of eastern Texas are destined to become deciduous forests of scrub oak or tall oaks, depending upon the degree of mesophytism. Such a state is remote under natural conditions, due to the effects of surface erosion which prevent these soils from maturing.

The ultimate climax type of the upland leached soils is more difficult to predict than that of the prairie. Pines are the pioneering trees, and remain as the dominant type where the soils are sufficiently sterile not to produce a floor covering which will shade out their seedlings. Pines, once established on sandy soil, tend to hold their dominance by their superior height and by their deep and extensive root competition. If conditions should become sufficiently fertile or mesophytic, pines would be crowded out by shading. At present the tall oak dominates in east Texas only on the slightly limy, fertile soils of the Greenville series and in certain better phases of the Orangeburg series. Pines dominate all leached soils, mainly the upland sands, westward to the limit of their climatic range. If the pine forest should become much more xerophytic, the post oak association would be the climax type. If conditions should become mesophytic, it is possible that the white oak-magnolia-beech-maple

association would come into dominance. The latter association is only incompletely expressed in the Willis area on hammock land near water courses, where transpiration is low and the summer temperature is reduced. It reaches a rather complete expression in the Big Thicket east of the Willis area. The pine forest under present conditions shows little tendency to develop a mesophytism that will enable the invasion of other forest types. Pine seedlings develop readily on the forest floor. Cut-over woodland returns to pine. This may be obscured at first by an open growth of young hardwoods, but in the course of about fifteen years pines have gained an ascendancy over the less rapidly growing hardwoods. The factors that operate to continue this dominance are: (1) the poverty of the soil which prevents shading out of seedlings; (2) the lack of a well developed layer of leaf mold, due to its destruction by microorganisms and frequently recurring ground fires; and (3) the structure of the soil that favors leaching and percolation of solutes favorable to deep feeding pine roots.

The low oak association, *Quercus minor* and *Q. marylandica*, is the climax type on all leached soils west of the pine forest and east of the shortgrass plains to the northwest and of the desert scrub to the southwest. This association is well stabilized and is gaining ground slowly as the Houston soils become more leached. This gain upon the prairie is not due to increased mesophytism but to leaching. The herbaceous vegetation of this association is more scanty and apparently as xerophytic as the associated prairies. This is the most stable and strikingly edaphic formation type of this region.

The mesophytic hardwoods dominate the stream valleys and certain other situations where the soils are somewhat fertile. Certain species show a tendency to extend their range into the pine forest, where conditions have become more mesophytic, and in these situations when pines are removed the hardwoods apparently remain dominant. This permanent supplanting of pines is infrequent in the upland soils of the Willis area. In general it can be said that the tall hardwood association is not only holding its own but probably gaining ground.

The retrogressive factors which influence succession are as follows: (1) fire, which aids in the dominance of pine forest on leached

soils and prairie grasses on unleached soils; (2) grazing, which favors pine and xerophytic oaks; (3) lumbering of pine, which encourages the dominance of hardwoods; (4) the reversion of old fields, once cultivated, to pine forest or prairie depending on the character of the soil.

Summary

This paper is an attempt to present data on the relative value of different factors operating to determine edaphic formations on certain soil types. In order to define the plant communities it has been necessary to divide them on the basis of leached or unleached conditions of the soil, on the influence of water and topography, and on the nature of soil texture and aeration. The valence of abundance has been estimated for most of the members of each community, and the probable steps in succession have been considered. The climax types of vegetation have been named, and some of the conditions producing them have been presented.

1. Soil texture and aeration as factors in plant distribution are expressed (a) in the vegetation and in the decided difference in the valence of frequency of each species with changes in soil texture; (b) by the occurrence of a number of species in dry, well aerated sands that are infrequent in dry poorly aerated leached clays; (c) by the presence of a number of silicoles common to alkaline and leached sands and infrequent upon clay soils; (d) by difficulty of root development in the subsoil of prairies of this area; and (e) by the intolerance of certain plants to destruction of lateral roots by soil cracking. Much of the difference in the structure of the vegetation of the respective soil types of this area is due to soil texture and aeration.

2. Water apparently is not the major controlling factor in determining the difference in the structure of edaphic formations under consideration, although it is an important factor in the xerophytic, semixerophytic, mesophytic, and hydrophytic divisions of edaphic formations. Evidence that water may be of minor importance is shown by the societies of winter annuals and weeds on cultivated soils which are little affected by the water differences. The conditions of leached upland forested ridges apparently are more xerophytic than those of less exposed prairies. The vegetation of the

former shows a higher fatality rate than that of the prairie. Neither transpiration nor soil water explains adequately the difference in xerophytic forest and prairie of eastern Texas.

3. The maturity of the soil as indicated by its chemical nature is a very important factor in plant distribution. Apparently it is the major factor in determining the edaphic formations of this climatic region, as shown by (a) characteristic weed societies for cultivated soils of unlike chemical nature yet similar in being well aerated and supplied with moisture; (b) occurrence of distinct winter annual societies on leached and unleached soils; (c) the absence of calcicoles upon leached plastic clay although frequent upon calcareous plastic clay; (d) the frequency of most silicicoles upon leached plastic clay and the infrequency of these on calcareous plastic clay; (e) the difference in the associations on calcareous and leached sand; (f) the lack of similarity in the hydro-mesophytic associations of acid swamps and alkaline swamps; (g) the presence of species common to alluvial and upland unleached soils but infrequent or absent on upland leached soils; (h) the qualitative and quantitative differences in microorganisms of soils of contrasting chemical characteristics.

4. In considering biotic factors it was found that (a) where bacteria are abundant, producing an accumulation of mild humus, soils become dark and productive, and where fungi are abundant decomposition becomes more complete and soils are gray or yellow and unproductive; and (b) grazing favors pine forest perpetuation and is unfavorable to prairie continuance.

5. Fire is very favorable to the perpetuation of prairie by the destruction of shrubs, small trees, and seedlings. Intermittent fires favor the dominance of pine forest by the destruction of forest litter, thus checking mesophytism and soil improvement.

6. The plant associations considered are the most definite where soil conditions offer the greatest number of distinctive chemical and physical characteristics, as when a limy Houston clay is associated with leached Norfolk sand. Of the 178 species frequent on Norfolk sand, 118 seldom occur on Houston clay; and of the 161 species frequent on Houston clay, 101 are infrequent on sand.

7. The plant associations are less distinct where they occupy soils which differ mainly in one of these groups of factors; yet this differ-

ence may be very striking where soils differ greatly in chemical nature, as in Susquehanna clay and Houston clay. Of the 163 species frequent on sour Susquehanna clay, 103 seldom occur on calcareous Houston clay. This difference is less marked where the factor differences vary widely physically, as in Norfolk sand and Susquehanna clay, than where they vary widely chemically. Of the 178 species frequent on Norfolk sand, 20 seldom occur on Susquehanna clay.

8. When two soils of only slight difference are associated, as in Tifton and Norfolk or Ruston and Orangeburg, it follows that the vegetational differences will be slight. These differences will be mainly in frequency of the plants.

9. The majority of plants show preference for certain combinations of edaphic factors. Few are ubiquitous to all soils in anything like an equal frequency.

10. Edaphic factors exert their minimum influence in mesophytic conditions but are quite marked in xerophytic and hydrophytic conditions. Mesophytic associations in the Willis area are very limited in their occurrence. The uplands are generally occupied by semixerophytic and xerophytic associations.

11. That edaphic factors may upset climatic expectations is shown in the occurrence of disjunct prairies in the forest region of central Alabama on Houston clay, and of disjunct pine forest in the prairie region of central Texas on Norfolk fine sand.

12. The six dominant associations represent in general six types in physiognomy; namely, the prairie, the low upland oaks, the tall upland oaks, the pine forest, the lowland hardwoods, and the mesophytic climax forest. (a) The prairies in this area are characterized by a factor complex as follows: a good supply of mineral nutrients (especially lime), a maintenance of grass competition for soil oxygen and moisture, a tolerance for the destruction of lateral roots by soil cracking, recurring fires, a soil texture that is unfavorable to deep or extensive root systems, and xerophytic conditions in late summer and autumn. The dominant grasses are *Andropogon saccharoides* var. *laguroides*, *A. furcatus*, *Sporobolus Drummondii*, and *Stipa leucotricha*. (b) The low oak association is characterized by sparse floor covering, which limits competition for soil oxygen and favors the penetration of oxygen to the subsoil, a lower content of clay in the

surface soil, lack of excess lime, and climatic xerophytic conditions. Dominant plants are *Quercus minor*, *Q. marylandica*, and *Hicoria glabra*. (c) The tall upland oak association is dependent on a medium supply of water and mineral nutrients. Dominant species are *Q. digitata* and *Q. velutina*. (d) The pine association is dependent on little competition, adequate water, illumination for seedling development, and a soil structure favorable to their root development. (e) The lowland hardwood association is characterized by an abundance of mineral nutrients and soil water. The dominant types are *Fraxinus americana*, *F. viridis*, *Ulmus americana*, *Quercus nigra*, *Q. phellos*, *Populus deltoides*, *Hicoria pecan*, and *Platanus occidentalis*. Lianas are frequent. (f) The mesophytic climax forest is characterized by abundant supply of water, low evaporation, low summer temperature, and infrequent occurrence of fires. This association is very limited in this area, but reaches a full expression just east of it in the Big Thicket, where the dominant plants are *Quercus alba*, *Liquidambar styraciflua*, *Nyssa sylvatica*, *Ilex opaca*, *Magnolia foetida*, *M. virginiana*, *Fagus americana*, *Acer saccharum*, and *Pinus taeda*.

13. The six dominant associations are all nearly stabilized, but there is some evidence that the prairies are losing ground slowly under the influence of leaching, and more rapidly under the influence of grazing and cessation of prairie fires, and that the pine forest is losing ground slowly under the influence of mesophytism.

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EFFECTS OF X-RAYS UPON GROWTH, DEVELOPMENT, AND OXIDIZING ENZYMES OF *HELIANTHUS ANNUUS*

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 360

EDNA LOUISE JOHNSON

(WITH ELEVEN FIGURES)

Introduction

The nature of the effects of X-rays upon seeds and seedlings is still in need of investigation. This paper deals particularly with the physiological effects resulting when various doses of X-rays are applied to seeds and young seedlings of *Helianthus annuus*. Few investigations up to this time have included observations continued until the end of the life cycle of the irradiated plants. When plants are subjected to changed conditions of any sort, there is a necessity for continued observation until maturity, before definite conclusions can be stated. In these studies, a sufficient number of plants were allowed to grow to maturity, so that it was possible to observe not only their immediate response to irradiation, but also any effects appearing during later development.

Results of various experimenters often have not agreed because the observations were made on different species of plants, or were carried out on seeds with varying water content, or employed various units of dosage. Some (25, 26) have observed acceleration of germination with weak irradiation, while a recent investigator (4) found none. Several (1, 24, 33) have reported acceleration of growth due to weak irradiation. Practically all have agreed that soaked or germinated seeds are more sensitive than dry resting ones. Experiments by the writer show that this is true also for seeds of *Helianthus*.

Very little has been done to show the relation of X-rays to abnormalities of form and to changes in internal structure. These subjects, together with a study of the physiological effects of X-rays upon respiration and the oxidizing enzymes, will be considered in this paper.

Materials and methods

The seeds used for the experiments were those of the so-called Russian sunflower, *Helianthus annuus*, from which the ovary wall was removed before irradiation. Air-dried seeds were found to contain 3.41 per cent water. Since others (1, 16, 21, 26) have found that the sensitivity to X-rays is affected by the water content of the seeds, this will be recorded in all cases.

The unit of dosage employed in the experiments to be described is the "human erythema" dose of IvY (19), and is designated by E. The "set-up" for one erythema dose is 60 K.V.M. (maximum kilovoltage), 5 milliamperes current, no filter, 30 cm. focal distance, 5 cm. portal of entry for 5.5 minutes.

For irradiation[†] the seeds were placed in open Petri dishes so arranged that the rays came vertically from above. Care was taken to treat the control seeds exactly as the irradiated ones, except for lack of exposure to the rays. Within a short time after irradiation, both irradiated seeds and controls were planted in 8-inch pots and all were placed under similar environmental conditions in the University of Chicago greenhouse. In most cases, ten seeds were used to a pot. After a period of from ten days to two weeks the plants were thinned, and only two were allowed to grow to maturity in a pot. Any exceptions to this procedure will be noted.

Since many (15, 16, 21, 26) have reported that plants differ in their sensitivity to X-rays according to the species, it was first necessary to determine the lightest amount of dosage which would affect the seed of *Helianthus annuus* in any manner, and also the smallest dose which would noticeably inhibit growth. The first was found to be between 1 E. and 5 E., while the latter was 10 E. Those seeds receiving heavy irradiation (20 E.) produced seedlings which died after developing to the point where a portion of the cotyledons appeared above the soil.

I. Action in causing fasciation

A very constant effect of X-rays on seeds and seedlings of *Helianthus annuus* is the production of fasciation in stems, leaves, and

[†] The X-ray equipment used was lent to Dr. A. C. IvY, formerly of the physiology department of the University of Chicago, by the Standard X-ray Company of Chicago, and by Dr. IvY placed at the disposal of the writer.

flowers. In the stems this is expressed by the flattened strap-shaped or ribbon-like expansion of the main stem. At the base the stem is generally cylindrical, but the apex may be combed or diffusely branched (fig. 1). The stems after becoming flattened usually show bifurcations or splittings somewhere along their length. The resulting branches sometimes remain unfasciated, but in many cases they themselves become divided again (fig. 2). Very often an indentation or groove, which becomes wider and wider as the stem flattens, runs up a portion of the stem.

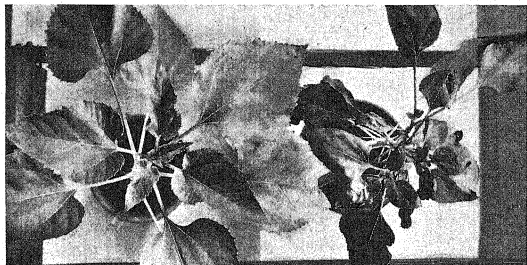


FIG. 1.—Left, control; right, tip of fasciated plant (48 days old) given X-ray dose of 5 E. when 4 cm. tall; viewed from above.

Fasciation is shown in leaves by changes in their number and position, and by changes in size and shape. Commonly the number of leaves is increased and phyllotaxy is distorted. Some of the leaves are normal, some are minutely notched at the apex, some are forked for one-third their length, and others are deeply forked. In extreme cases an original single leaf is split into two independent leaves, attached at the same point on the main stem (fig. 3). Many of the leaves in the growing tip show incurling and ruffling of the margins.

Fasciations of the flower were present in various forms; fusion sometimes took place in the involucrel region, giving the appearance of twin heads (fig. 4). In other cases the flower stalk forked a short distance below the insertion of the flowers and two distinct heads were observed.

Investigations of others (13, 18, 22, 23, 31, 39, 42) suggest that fasciation may be due to: (1) suitable conditions for rapid development; or (2) sudden arrest of the activity of the growing point due to insect or fungal attacks; or (3) mechanical injury to the growing point.

There is no doubt that fasciation is induced by the action of X-rays on seeds and seedlings of *Helianthus*. This has been proved by the writer in repeated experiments. Seeds containing 57.3 per

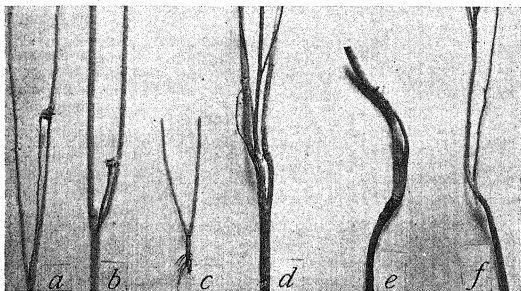


FIG. 2.—Types of fasciated stems from: *a*, plant whose seed had received dose of 7 E.; *b*, seed which had received dose of 10 E.; *c*, seed which had received 10 E.; *d*, seedling which had received 5 E.; *e*, seedling which had received 5 E.; *f*, seed which had received 8 E.

cent moisture were put into germinators for about 17 hours and then placed in short cylindrical packets of moist sphagnum. After three or four days' growth, the young seedlings were exposed to a dose of 5 E. The focal distance (30 cm.) was measured from the growing tip of the stem. The packets were then placed in soil without disturbance to the young seedlings. During early stages of growth of the seedlings and before fasciation appeared, the effects of irradiation were similar to those seen in young plants whose seeds had been irradiated. Aside from checking of growth, the first striking effect was noted in the abnormally shaped young foliage leaves with their pitted or pebbly appearance. The effects were seen only in

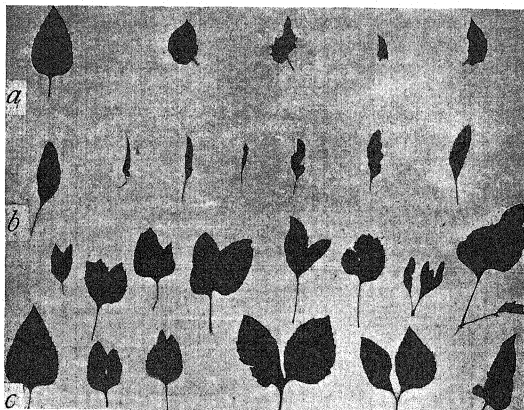


FIG. 3.—First leaf of each row, control; *a*, typically deformed leaves from upper regions of irradiated plants; *b*, abnormally ligulate leaves from lowest nodes; *c*, incipient fasciation shown by leaves.



FIG. 4.—Fasciated flower head produced on plant irradiated in young seedling stage.

foliage leaves, never in cotyledons. Table I shows the course of development of the young plants. The dates given for time of blossoming are from appearance of first flower to appearance of last. Height is taken from base of stem to highest growing point.

In other experiments by the writer, clear cases of fasciation were induced also in tomato and cosmos seedlings by means of X-rays. Various abnormalities appeared, as flattened stems, dichotomously branched main stems and lateral branches, and altered phyllotaxy.

TABLE I

FASCIATION, DECREASED TOTAL GROWTH, AND EARLY BLOSSOMING SHOWN AS RESULTS FROM IRRADIATING YOUNG SEEDLINGS WITH A DOSAGE OF 5 E.

SERIES NO.	HEIGHT AT TIME OF IRRADIATION (CM.)	HEIGHT AT TIME OF BLOSSOMING	TOTAL DAYS OF GROWTH BEFORE BLOSSOMING	WEIGHT AT MATURITY (GM.)		EVIDENCE OF FASCIATION
				Green	Air-dry	
KS. 3a...	4	198	119	108.9	Dichotomous branching 17 cm. from base of stem (greatly flattened)
KS. 3b...	4	135	101-113	72.5	Dichotomous branching 50 cm. from base of stem; 6 branches formed, all blossomed
KS. 3c...	4	124	101-112	21.5	First dichotomous branching at height of 77 cm.; further splitting of stem until 6 tips evident; 6 blossoms, one double
KS. 3d...	Only arch of hypocotyl showing	152	107	79.2	No abnormalities
KS. 3e...	Only arch of hypocotyl showing	138	104	17.1	Large double blossom due to splitting in involucre region (fig. 4)
Control...	5	144	112	26.4	Normal one-stemmed plants with single blossom
Control...	Only arch of hypocotyl showing	169	101	94.8	Normal
Control...	Arch of hypocotyl showing	171	127	Normal

In the experiments with *Helianthus* here described, fasciation was induced in 80 per cent of the cases by irradiating seedlings with one dose of 5 E. Experiments described later will record fasciations induced when slightly heavier doses were given to soaked seeds.

II. Proportional effect of dosage and growth

1. IRRADIATED SEEDS.—When seeds with a water content of 58.7 per cent were exposed to dosage ranging from 5 to 10 E., the amount of injury produced during the first three weeks of growth was approximately proportional to the dose. This effect proved to be transitory, and by flowering time the growth of the irradiated plants was almost equal to that of the controls.

Fasciation was present in some of the plants of this series. Fasciation of the stem was induced in 75 per cent of the plants whose seeds received a dose of 7 E.; those whose seeds received 8 E.

TABLE II
ABNORMALITIES PRESENT IN SEEDLINGS; SOAKED SEEDS IRRADIATED
WITH DOSAGE DESIGNATED

DAYS AFTER IRRADI- ATION	APPEARANCE AFTER RECEIVING X-RAY DOSAGE OF					
	5 E.	6 E.	7 E.	8 E.	9 E.	10 E.
13.....	Some leaf distortion throughout entire series Dark and light patches of green giving mosaic appearance Growth of tip inhibited					
29.....	Stem diameter shows considerable increase above first node					
				Dichotomous branching	25% plants dead	50% plants dead; fasci- ation of main stem
43.....	Slight abnor- mality of leaves	Young leaves still show- ing decided abnormali- ties	Marked ab- normality in leaves; one plant showing three tips; one, two tips	Abnormal phyllotaxy and leaf de- velopment; plant with three tips	Leaves curled; ax- illary buds developing in one plant	Abnormal phyllotaxy
50.....	Almost nor- mal	Few abnor- mal leaves at top	75% fasci- ation	50% fasci- ation	See above	Fasciation in both stems
74.....	Apparently normal ex- cept for pe- culiar ap- pearance of basal inter- node	Slight leaf ab- normality; peculiar ap- pearance of basal inter- node	See above	See above	Leaves large but unsym- metrical; abnormal phyllotaxy	See above
96 (time of blossom- ing).....	Plants and flowers al- most nor- mal	No fasci- ation; al- most nor- mal	Best fasci- ation shown in stem split 62 cm. from base	Best fasci- ation in stem with secondary and terti- ary split- ting; three small blos- soms on this plant	No fasciation of stems	One plant with split 87 cm. from base of flat- tened stem; two blos- soms pres- ent; other plant with split 23 cm. from base; two normal and one stunted blossoming stem

showed fasciation in 50 per cent of the cases. Of the plants whose seeds received 10 E., all of those that grew to maturity showed fasciation. The type of fasciation in these plants was that of dichoto-

mous branching, where the main stem split up into secondary and tertiary branches. The earliest effect noted in the seedlings of ir-

TABLE III

TOTAL HEIGHT OF IRRADIATED SEEDLINGS MEASURED AT WEEKLY INTERVALS

SERIES NO.	HEIGHT IN CM. AT WEEKLY PERIODS									
	1	2	3	4	5	6	7	8	9	10
OS .35	Dose of 5 E.									
1....	2.0	4.4	7.8	18.0	36.0	52.0	66	78	93	105
2....	2.2	4.5	10.2	22.0	44.0	52.0	68	83	98	108
3....	2.0	5.7	12.5	25.5	46.0	57.0	72	84	97	111
4....	2.0	6.0	11.8	25.5	46.0	59.0	72	84	99	112
5....	2.0	4.2	7.8	18.0	32.0	43.0	54	63	75	86
6....	4.0	8.0	15.2	23.0	41.0	56.0	66	74	90	103
7....	2.5	6.0	11.7	20.0	37.0	54.5	68	76	95	111
8....	2.2	4.6	11.4	23.0	41.5	54.5	64	78	95	105
9....	3.0	5.4	9.8	19.0	37.0	50.0	62	78	90	110
10....	3.0	5.2	9.0	18.6	30.0	36.0	40	48	62	70
11....	2.0	3.2	7.0	17.0	38.0	36.0	71	85	99	103
12....	3.0	4.6	7.0	13.0	23.0	32.5	37	40	60	62
Average.	2.5	5.15	10.1	20.2	37.6	48.5	61.7	72.5	95	99
OS .310	Dose of 10 E.									
1....	1.0	Died								
2....	0.5		4.2	11.0	20.0	49	61	74	90	107
3....	0.3	3.0	4.4	8.0	10.5	15	26	39	58	78
4....	0.1	2.0	7.2	14.5	20.1	39	50	64	82	98
5....	0.1	2.7	4.7	8.0	27.0	38	54	59	66	74
Average.	0.17	2.4	5.1	10.4	19.4	35	48	59	74	89
	Control									
1....	2.0	7.0	15.0	28	49	72	84	91	103	115
2....	3.0	6.8	12.6	24	40	57	65	75	89	92
3....	2.2	6.6	14.0	24	48	68	75	87	95	98
4....	1.0	4.5	17.0	23	40	62	84	92	100	117
5....	3.0	6.6	15.0	31	49	67	77	88	104	115
6....	1.0	4.4	12.5	24	46	67	75	98	105	115
7....	2.0	4.6	10.5	25	41	69	74	81	93	99
Average.	2.0	5.8	13.8	25.6	45	66	76	87	98	107

radiated seeds was the peculiar mosaic appearance of the young foliage leaves as they unfolded. This seemed to be due to the irregularity of the amount of green color in the leaves. As they became

older the distribution of chlorophyll became normal. Abnormalities in shape of leaves from lower nodes tended to disappear as the plants grew older. Table II records the appearance of plants of this series at certain intervals.

2. IRRADIATED SEEDLINGS.—The growth of irradiated seedlings throughout their development appears to be almost inversely proportional to dosage. Seedlings were prepared for irradiation by the

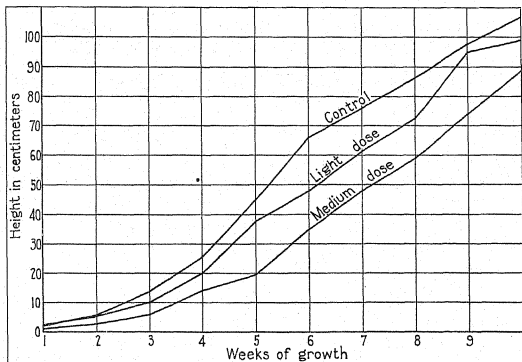


FIG. 5.—Curves showing total growth of control plants and those irradiated in seedling stage with light and medium doses; growth measured at weekly intervals.

method described, and when approximately 2 cm. in height one group was given a dose of 5 E., the second group was given a dose of 10 E., and the third group was used as a control. The experiment was closed at the end of 65 days when all the control plants had bloomed. Very warm days during the growth period (July 8–September 11) probably account for the very short period of blooming. The delay in blossoming seen in seedlings receiving 10 E. is thought to be due to the pronounced stem fasciations present. Repeated divisions of the main stem had resulted in the presence of many branches. At the close of the experiment practically all of these bore buds. The weekly total heights of individual plants of

each group are recorded in table III, together with the average for the groups. Fig. 5 represents the curve of growth based on the average growth for each group. In table IV are given brief descriptions of abnormalities occurring in irradiated seedlings. In those receiving 5 E. fasciation occurred in 66 per cent of the plants, in those receiving 10 E. it occurred in 100 per cent.

TABLE IV
EFFECT OF IRRADIATION UPON YOUNG SEEDLINGS

SERIES NO.	DOSE	CONDITION AFTER 65 DAYS		ABNORMALITIES
		Average height in cm.	Per cent in blossom	
OS .35.....	5 E.	98	66.6	Altered phyllotaxy (three or four leaflets in whorl); leaves spotted and pitted apparently from unequal distribution of chlorophyll; lower leaves ligulate and narrowly lanceolate Types of fasciation: (1) incipient fasciation of leaves similar to that shown in fig. 3; (2) flattened stems; (3) dichotomously branching stems which again divide; (4) double head
OS .310.....	10 E.	67	None	Pronounced fasciation in all plants; stems flattened, grooved; and dichotomously branched; plant shown in fig. 7 (right) divided into branches <i>A</i> and <i>B</i> . <i>A</i> remained unbranched; <i>B</i> divided into one short branch (<i>a</i>) and one long branch (<i>b</i>), <i>b</i> split into three branches (one with tip with four buds, other tips had one bud each). Close of experiment at end of 65 days may have prevented further fasciation
Control.....	107	100	None

This experiment indicates that a dose of 10 E. causes very severe injury to plants when they are irradiated in the young seedling stage. Fig. 6 shows appearance of some of these plants sixteen days after irradiation. The cotyledons appear unharmed but the young foliage leaves as they develop show much distortion. After a period of about three weeks there is a partial recovery from injury, and from that time on more normal growth is made. Fasciations were evident twenty-one days after irradiation; these became more pronounced as the plant developed (fig. 7). Here, as in the experiments just preceding, we have evidence that if the plant is not killed by the dose

given, it will partially recover and a normal increment of growth will be made until the plant approaches the height of the controls.

III. Effect upon catalase activity

Since some (14, 17, 36) have felt it reasonable to assume that catalase activity can be considered an index to metabolism, experiments were undertaken to ascertain whether inhibition of growth which accompanies heavy irradiation is associated with decreased

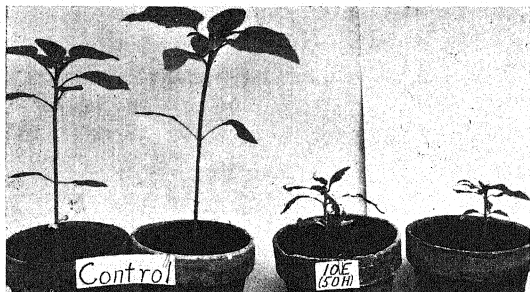


FIG. 6.—At right, two 20-day old seedlings irradiated with 10 E. when less than 1 cm. in height (appearance typical for this age); partial recovery from effects of irradiation took place after photograph was taken, and plants eventually reached a height of 67 cm. in 63 days; controls averaged 107 cm. in same period.

catalase activity. The one experiment (37) found in the literature which deals with the catalase activity of X-rayed plants, states that the activity of the sweet almond is not affected by irradiation. Those (28, 37) experimenting with the catalase of liver have found that irradiation diminishes its activity.

The writer has employed an apparatus modified from that of APPLEMAN (5). Throughout the course of the experiments the water bath was kept between 19°–20° C., and all results were calculated to standard conditions of temperature and pressure. The commercial form of 3 per cent Oakland dioxygen, frequently tested by the potassium permanganate method, was used. During the course of

the experiments the plant tissue and dioxygen were kept alkaline by the addition of decinormal NaOH and calcium carbonate. In one group of experiments the plant material used was powder, prepared by drying seedlings in the laboratory for seven days, then grinding and sifting the powder through an 80-mesh sieve. In the second group of experiments described, green tissue was ground in a mortar

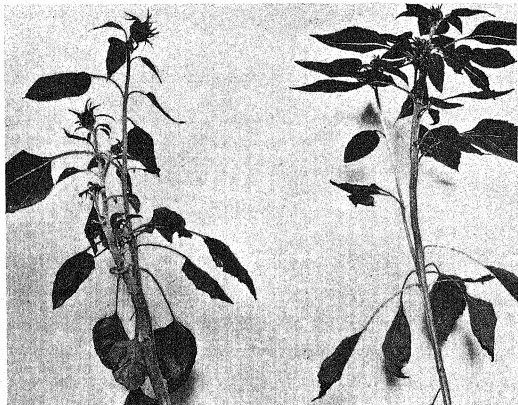


FIG. 7.—Fasciated sunflowers produced by irradiating 5-day old seedlings with 5 E. (left) and 10 E. (right); strap-shaped condition of stem apparent in both specimens.

with an excess of calcium carbonate, and then washed quantitatively into the shaking bottle with 15 cc. of distilled water. Care was taken to grind the tissues each time to a comparable state of fineness. When the bottle with its contents had reached the temperature of the bath, 5 cc. of dioxygen was added and the whole shaken for ten minutes. The majority of determinations were run in triplicate, the numbers reported being the average of these determinations. Whenever possible a small enough amount of plant material was used, so that 18–20 cc. of oxygen was liberated in ten minutes, since recent

experiments (32) have shown that this adjustment gives the most satisfactory results.

1. CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS EXPOSED TO 4, 10, AND 15 E.—There is a depression of catalase activity in seedlings whose seeds have been exposed to the action of X-rays. This is less marked in older seedlings and is accompanied by greater growth. In cases cited earlier in this paper, doses not causing death of a plant usually showed only transitory effects upon growth. Table V gives the results of catalase determinations made with dried powder of seedlings grown for the stated number of days, and then

TABLE V

CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS IRRADIATED WITH 4, 10, AND 15 E.; DETERMINATIONS MADE ON SMALL QUANTITIES OF AIR-DRY POWDER

DOSE	CONDITION OF SEED	AGE OF PLANT (DAYS)	WEIGHT OF POWDER IN (GM.)	CC. OF O ₂ LIBERATED IN MINUTES					O ₂ PER GM. AIR-DRIED WEIGHT (CALCULATED)
				1	2	3	5	10	
4 E.	59% water	5	0.015	8.6	13.0	16.9	20.2	25.01	1840
Control..	50% water	5	0.015	14.2	17.7	22.3	28.2	35.06	2337.33
15 E.	Air-dry	5	0.015	9.7	14.6	17.7	21.4	25.55	1704
Control..	Air-dry	5	0.015	11.8	18.7	22.32	27.0	31.89	2126
10 E.	56.5% water	13	0.01	8.27	13.51	16.09	19.96	21.55	2155
Control..	56.5% water	13	0.01	10.89	16.66	20.00	24.27	29.28	2928

prepared as already stated. The 5-day old seedlings were grown in sphagnum, while those given a dose of 10 E. and examined at the end of thirteen days were grown in soil. The latter seem to have recovered from the effects of the dose, since the catalase activity became almost equal to that of the control.

2. CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS EXPOSED TO KILLING DOSE.—Soaked seeds with a water content of 58.7 per cent were exposed to a dosage of 40 E. Seedlings from these developed to a total height of 1-3 cm. They remained green for some time without increasing in size, and then died. Seedlings for this experiment were grown in sphagnum, and at intervals, as stated in the experiments, were used in determining catalase activity. The 53-hour seedling was found to contain too much catalase for the entire seedling to be used in the determination, hence one-half or one-

fourth of the seedling, containing all representative parts, was used in all determinations except the first one. Oxygen liberated per gram absolute dry weight was used as a basis for comparison of the catalase activity of seedlings selected at successive intervals of 48 hours, and controls of the same age. Table VI gives results from this series of experiments, and fig. 8 gives diagrams representing determinations made. The activity of the control is represented by clear rectangles, while the black rectangles show determinations made on irradiated material.

TABLE VI

CATALASE ACTIVITY OF SEEDLINGS FROM SEEDS RECEIVING A VERY HEAVY DOSE (40 E.); DETERMINATIONS MADE AT 48-HOUR INTERVALS

AGE AND LENGTH OF SEEDLING	WEIGHT OF GREEN MATERIAL (GM.)	CC. OF O ₂ LIBERATED IN MINUTES					O ₂ PER GM. GREEN WEIGHT (CALCULATED)	O ₂ PER GM. ABSOLUTE DRY WEIGHT (CALCULATED)
		1	2	3	5	10		
5-hour seedling . . .	0.0688	2.6	5.1	7.1	10.0	14.2	206.35	324.7
Control	0.0606	1.8	3.4	4.8	6.7	8.2	135.3	210.7
53-hour seedling . .								
(1 cm.)	0.019	5.5	10.0	13.5	17.8	23.5	1236.84	3283.0
Control (2.5 cm.) . .	0.027	11.4	13.7	21.9	26.2	29.8	1103.7	4405
101-hour seedling								
(2 cm.)	0.0325	7.8	13.0	16.7	22.1	27.0	858.5	3430
Control (12 cm.) . .	0.1051	17.0	29.1	36.5	49.5	51.8	494.76	3320
149-hour seedling								
(2.4 cm.)	0.0541	3.74	7.2	9.6	13.3	18.7	534.6	1540
Control (18 cm.) . .	0.0966	7.5	12.7	17.5	25.9	29.5	8220.1	2106
177-hour seedling . .								
(2.6 cm.)	0.0866	5.0	8.4	10.8	14.73	21.15	244.2	1572
Control (21 cm.) . .	0.1921	5.6	9.4	10.8	16.8	23.3	121.3	2369

The preceding experiments showed consistently lower catalase activity in irradiated material than in the control seedlings. It may be concluded, therefore, that irradiation causes a depression in catalase activity.

IV. Effect on respiration

After finding decreased catalase activity in seedlings whose seeds had received a heavy dose of X-rays, one would naturally inquire as to the effect of irradiation of seeds upon respiration of their seedlings. No previous experimentation seems to have been done with the respiration of irradiated plants. One investigator (7) found that canaries used an increased amount of oxygen immediately after ex-

posure to X-rays, but after one hour metabolism was much lower than it had been before.

In the two following experiments, comparisons were made between respiration of normal seedlings and seedlings of the same age whose seeds had been exposed to heavy doses. The amount of CO_2 liberated by the seedlings was used as an index to respiratory activity.

Helianthus seeds having a water content of 58.7 per cent were exposed to a dose of 40 E. A few hours after irradiation, they were placed on moist cotton in a type of respirometer devised and described by OTA (30). By means of this apparatus only CO_2 -free air is admitted to the respiratory chamber. The atmosphere of the chamber is exposed to normal NaOH , which occupies a tube placed just below the respiratory chamber. The respirometers were placed in a

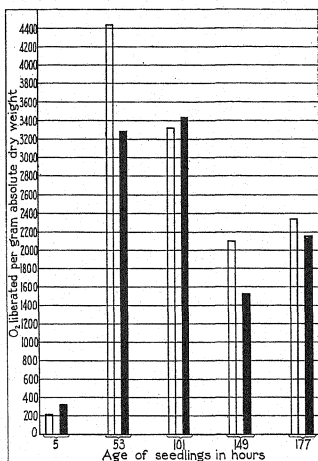


FIG. 8.—Diagrams representing catalase activity of seedlings from heavily irradiated seeds (black rectangles) as compared with that of controls (clear rectangles); diagrams based on data in table VI.

Freas water bath, the temperature of which was kept at 20° C. After each 24-hour period, the amount of CO_2 given off by the seedlings was determined by the double titration method of BROWN and ESCOMBE (8). Table VII shows the average results, from two series of determinations, of the amount of CO_2 expired from twenty seedlings.

Seedlings from heavily irradiated seeds apparently lack the ability which an ordinary seedling has to take up water. It seems possible

that the irradiation affects the permeability of the membranes in such a way that absorption is retarded. Absolute dry weight determinations showed that seedlings from irradiated seeds had a much higher percentage of dry weight than the controls. For this reason a

TABLE VII
AMOUNT OF CO₂ LIBERATED DAILY PER 20 SEEDLINGS
FROM SEEDS DOSED WITH 40 E.; CO₂ FROM
CONTROLS ALSO GIVEN

AGE OF SEEDLINGS (DAYS)	MILLIGRAMS OF CO ₂ PER 20 SEEDLINGS PER DAY	
	Irradiated	Controls
1.....	14.3	18.1
2.....	23.2	26.5
3.....	24.2	28.32
4.....	23.1	27.5
5.....	22.2	37.0
6.....	22.2	35.2
7.....	22.0	30.0
8.....	22.0
9.....	17.6	25.3

determination was made of the amount of CO₂ expired per gram of absolute dry weight. Seed treatment was as described, except that the dose given was 24 E., an amount sufficient to stop growth after a length of 1-3 cm. had been attained. Average results from two series of experiments are recorded in table VIII.

TABLE VIII
AMOUNT OF CO₂ EXPIRED BY SEEDLINGS FROM
IRRADIATED SEEDS

AGE OF SEEDLING (HOURS)	MILLIGRAMS CO ₂ PER GM. ABSOLUTE DRY WEIGHT PER HOUR	
	Irradiated	Control
78.....	1.742	3.043
102.....	3.369	4.317

These results indicate that depressed respiration accompanies inhibition of growth caused by a dosage of X-rays varying from 24 to 40 E. Normally seedlings which are deprived of a proper food supply lose considerable dry weight through the oxidation processes. That

seedlings from irradiated seeds showed a higher percentage of dry weight present would indicate that oxidation was being retarded and the CO_2 output decreased. Others (6, 12, 35) have found parallel behavior between catalase activity and respiration in normal seedlings. Results here offered give added proof of correlation between the two processes, for both are depressed by X-rays.

V. Effect on oxidase activity

Oxidase activity was neither depressed nor increased in seedlings from irradiated seeds. These results may be contrasted with those of BUNZELL (10, 11), who found that seedlings dwarfed by under or over supply of water had oxidase activity increased.

A study was made of leaves which showed anomalies of form resulting from irradiation. These did not exhibit increased oxidase activity as do leaves showing physiological disturbances due to diseases. Abundant evidence (10, 11) has been found of increased oxidase action in leaf tissue of plants in cases of mosaic disease of tobacco, curly top of sugar beet, and diseases of spinach. WELS (38) believes that the growth-checking effects of X-rays are not due to an influencing of oxidation processes in cells of yeast, bacteria, and some animal cells. Others (27) have found that the oxidase system of leucocytes was not influenced by the rays.

1. OXIDASE ACTIVITY OF ENTIRE SEEDLINGS FROM HEAVILY DOSED SEEDS.—Oxidase activity was found to be unchanged in seedlings from seeds which had been irradiated with a dose of 24 E. Seeds with a water content of 58 per cent were exposed to that dose. After growth for five days in sphagnum, the young plants were dried before an electric fan, powdered to pass through a sieve of 80 meshes to the inch, and the powder then thoroughly dried in a vacuum desiccator over calcium chloride.

Determinations were made in the simplified apparatus described by BUNZELL (9), the principle of which involves the measurement of the rate of oxygen absorption as determined by measuring changes of pressure within the reaction flask. The oxidizing agent employed was 1 per cent pyrocatechol. The rate of shaking was five complete revolutions in approximately three seconds. A definite amount of dried powder was placed in the long arm of the apparatus by means of a funnel and a camel's hair brush. To this 5 cc. of distilled water

was added; 1 cc. of 1 per cent pyrocatechol was added to the short arm. The apparatus and its contents were allowed to stay at the temperature of the experiment for thirty minutes, before the apparatus was closed and shaking begun. Table IX shows the results obtained with use of absolutely dry powder from plants whose seeds had received a dose sufficient to cause death of seedlings within a few days after germination. The table shows that there is no significant difference between the activity of the irradiated material and that of the control.

2. OXIDASE ACTIVITY OF ABNORMAL LEAVES FROM IRRADIATED SEEDS.—The material for use in this experiment was collected from

TABLE IX
MANOMETER READINGS OBTAINED BY USE OF 0.02 GM. POWDER
FROM 5-DAY SEEDLINGS WHOSE SEEDS HAD RECEIVED DOSE
OF 24 E.; DURATION OF EXPERIMENT 1.5 HOURS

NO. OF DETER- MINATION	TEMPERATURE (°C) AT TIME OF MEASUREMENT	MANOMETER READINGS EXPRESSED IN CM. OF MERCURY	
		Plants from irradiated seeds	Normal plants
I.	28.0	2.7	2.6
II.	28.0	2.4	2.4
III.	28.5	2.55	2.5
IV.	28	2.3	2.3
V.	28	2.3	2.3

plants whose seeds had been irradiated with doses light enough to avoid death. Leaves showing incipient fasciation, or which were abnormal in other ways, were collected, dried, powdered, and stored in a desiccator. Control leaves were treated in the same manner. Table X shows results of these determinations, and again only a slight difference is apparent in oxidase activity of abnormal leaves and that of control leaves.

From the data presented in tables IX and X, it is evident that inhibition of growth is not always, as has been maintained, accompanied by increased oxidase activity. There is approximately no difference in the oxidase activity of equal quantities of dry powder derived from retarded seedlings and that from control seedlings; neither does the oxidase activity of leaves deformed by the action of X-rays differ materially from that of normal leaves.

Oxidase appears to be an exceedingly stable system, and an amount of irradiation which inhibits growth after the seedling has attained a height of 1-2 cm. does not affect oxidase activity.

VI. Histological investigation

1. STEMS OF MATURE PLANTS AND OF 9-DAY OLD SEEDLINGS.—

One character which was most noticeable in mature plants from irradiated seeds, and never apparent in the controls, was the roughened, dark brown appearance of the stem in the hypocotyl region. When examined microchemically, it was found that the epidermis of the irradiated material, which was broken and irregular in outline, contained considerable suberin, while little or none was present in the control.

TABLE X

MANOMETER READINGS OBTAINED BY USE OF 0.02 GM. OF POWDER
FROM ABNORMAL LEAVES OF IRRADIATED SEEDS;
DURATION OF EXPERIMENT 1.5 HOURS

NO. OF DETER- MINATION	TEMPERATURE (°C) AT TIME OF MEASUREMENT	MANOMETER READINGS EXPRESSED IN CM. OF MERCURY	
		Leaves showing anomalies	Normal leaves
I.	27.50	0.50	0.45
II.	28.70	0.45	0.60
III.	28.75	0.40	0.50

Cross-sections were cut through the hypocotyl region of mature plants whose seeds had received doses of 8, 9, and 10 E. respectively. These were made midway between the emergence of the first lateral root and the point of attachment of the cotyledons. When compared with cross-sections of the control in the same region, there was noted a great increase in the amount of xylem in the irradiated specimens, and a corresponding decrease of pith cells. Table XI shows measurements of cross-section of stem taken midway between the emergence of the first lateral root and the point of attachment of the cotyledons. Each measurement given is the average of three taken on the same section. Measurements are recorded from sections of two control plants, and from two plants whose seeds were subjected to a dose of 8 E. Of those given 9 and 10 E., only one plant each was used for measurement of sections.

From results given, it is seen that while there is no appreciable increase in the amount of cork in the control stem, the corky layer of the irradiated material occupies 8-10 per cent of the whole width. The cortex, which in the control stems makes up approximately 22 per cent of the diameter of the section, occupies only 9.5-18.7 per cent of the whole in stems whose seeds were X-rayed. There is a marked difference in the amount of xylem present in the irradiated material and in the control stems. In the former the xylem constitutes 57.7-69.8 per cent of the total diameter, but only about 38

TABLE XI

MEASUREMENTS OF CROSS-SECTION OF STEM TAKEN MIDWAY BETWEEN EMERGENCE OF FIRST LATERAL ROOT AND POINT OF ATTACHMENT OF COTYLEDONS; EACH MEASUREMENT IS AVERAGE OF THREE TAKEN ON SAME SECTION

DOSE	WIDTH OF PITH IN μ	PERCENT-AGE OF WHOLE	WIDTH OF XYLEM IN μ	PERCENT-AGE OF WHOLE	CORTX IN μ	PERCENT-AGE OF WHOLE	CORK LAYER IN μ	PERCENT-AGE OF WHOLE
Control (a)	3066	37.2	1566	38.0	1017	24.8
Control (b)	3417	41.4	1600	38.7	817	19.8
Average	3241	39.3	1583	38.3	917	22.3
8 E. (a)	1066	11.3	3217	68.6	567	12.1	367	8.0
8 E. (b)	907	8.4	3833	71.1	650	12.0	450	8.5
Average	986	9.8	3525	69.8	608	12.0	408	8.2
9 E.	1133	16.6	2217	65.0	325	9.5	300	8.9
10 E.	10000	13.7	2100	57.7	683	18.7	354	9.9

per cent in the control. In control stems, the diameter of the pith is at least three times that of stems whose seeds received a moderate amount of irradiation. Others (1, 29) also have noted the increase of xylem and cork and the decrease of pith which accompanied irradiation. Fig. 9 shows diagrammatically the relative proportions of the various regions of the stem in half sections taken from a control hypocotyl, and from a seedling whose seed had received a dosage of 10 E.

The character of the elements present in the cross-sections of the stem differs in irradiated material from that of the control. In the former the pith cells from the same regions are much smaller in diameter, and are slightly thicker walled. The irradiated material has smaller and more compactly arranged xylem cells. Fig. 10 shows

drawings of a few cells from the center of the pith regions of stems which are being compared. Fig. 11 represents the protoxylem and a portion of primary xylem in the hypocotyl region of a mature control plant, and of one whose soaked seeds received a dose of 10 E. The wood elements in the irradiated specimens are seen to be of smaller dimensions than are those of the control.

Paraffin sections of hypocotyl of seedlings nine days old were examined to find whether plants derived from seeds which had been exposed to a dose of 10 E. showed any differences in their anatomical structures from controls of the same age. The most striking differences seemed to be in the amount and character of the xylem. The irradiated material had a very marked increase in the number of xylem cells. Instead of showing fibrovascular bundles somewhat oval in shape, as is characteristic of bundles in young control stems,

the xylem region of each bundle was much extended in width (tangentially). The number of distinct bundles in the control stems examined was ten. The xylem varied in width from 80 to 130 μ . The stem of the irradiated material, however, showed but seven distinct fibrovascular regions. In one portion the xylem tissue appeared as a continuous mass, and looked as if three or more vascular bundles had fused. The individual areas of xylem varied in width from 80 to 560 μ . The total circumferential width of xylem of the

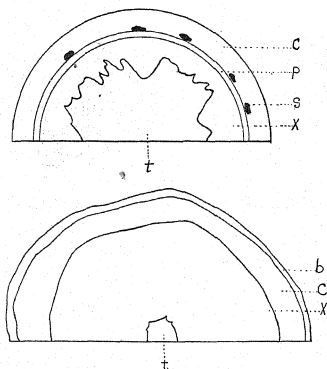


Fig. 9.—Diagram of transverse section of stem through hypocotyl region of mature plant: above, control; below, from plant whose seeds had received dose of 10 E. (b, cork; c, cortex; s, hard bast; p, phloem; x, xylem; t, pith); phloem of irradiated material not distinguishable, as it was injured by pressure of xylem; no cork formed in control stem.

hypocotyl from irradiated material amounted to $1530\ \mu$, a width approximately 45 per cent greater than that of control tissue.

The individual xylem cells in the hypocotyl of irradiated specimens showed a great similarity of size. In the control small cells measured approximately $5 \times 7\ \mu$, the largest cells $40 \times 35\ \mu$. In the

irradiated material the cells were of more uniform size. The average of many cells was approximately $13.5 \times 11.5\ \mu$.

2. RADICLES OF 6-DAYS OLD SEEDLINGS.—The diameter of the control radicle at a distance of $200\ \mu$ from the base of the root cap was $400\ \mu$ in diameter. The appearance of the cells was similar to that of the usual young root cells. The shape was rectangular; the cells, practically all of the same size, were thin walled and well filled with protoplasmic contents. The nucleus was large in proportion to the size of the cell. Cells in this meristematic region were measured, and the average size of ten cells found to be approximately $13.5 \times 15\ \mu$.

The radicle from irradiated

material was only about one-half as thick as the control root. The cells in a region comparable with that studied in the control showed an entirely different appearance. They indicated that all growth had ceased, and the cells seemed to have reached the stage of cell enlargement. The cells were elongated; many of them had lost their protoplasmic contents entirely, others showed great vacuolation. KOMURO (26) records similar results from his study of the root tip of *Vicia Faba*. In marked contrast to the meristem of the control, where in these preparations a nucleus was evident

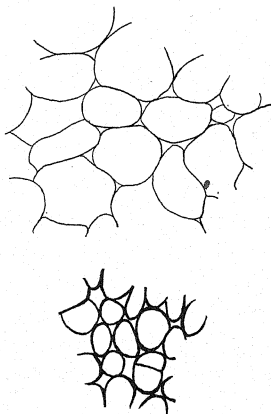


FIG. 10.—Cells from center of pith region of mature stem: above, control; below, from plants whose seeds had been irradiated with dose of $10\ \text{E.}$; $\times 150$.

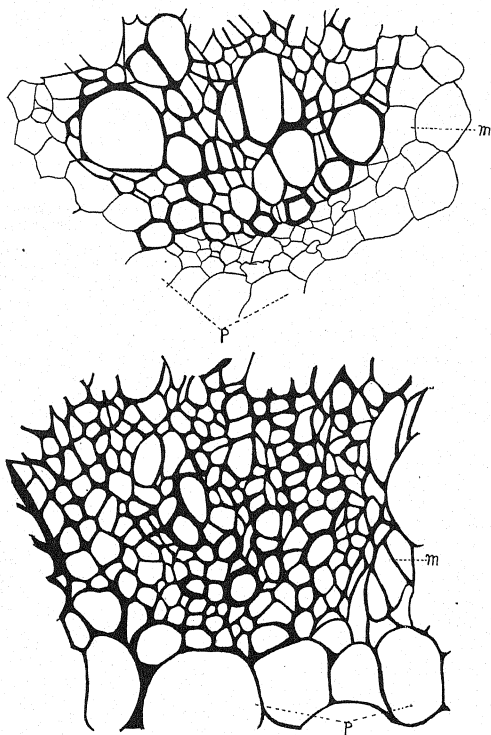


FIG. 11.—Above, primary xylem from mature stem of control (*p*, pith; *m*, medullary ray cells); below, primary xylem from mature stem of plant whose seed had received dose of 10 E. (note smaller dimensions of wood elements, thickened walls of pith cells (*p*), and of medullary ray cells (*m*), as compared with those of control stem); $\times 600$.

in every cell, the meristem of the radicle of the irradiated material showed but an occasional nucleus. In the same region of the root as that examined in the control, the average size of ten cells measured was $26 \times 46 \mu$, or twice the cross-section area of the control.

Discussion

In this investigation the plants were allowed to grow to maturity, so that not only the immediate influence of irradiation was noted, but any later after-effects as well. In summarizing the results of investigators who have studied the influence of X-rays on seeds and seedlings of various kinds, GELLER (15) made the criticism that most of the observations were not carried on long enough to determine whether the effect on plants is merely a transitory one, or whether it also exerts an influence on the total amount of growth.

Preliminary experiments showed that no immediate visible effects resulted when plants were given a light dose (4 E. and below). These plants at maturity, nevertheless, were shorter and weighed less than the controls. The period required for blossoming was also shortened. When a heavy dose (20-30 E.) was given, growth stopped after the hypocotyl had developed to an average length of 1.5 cm. The tip of the root showed a brown coloring, and, in the great majority of cases, no sign of a growing stem tip was visible.

Germination of seeds is not hastened by irradiation, but the percentage of seeds which germinate is slightly reduced. Irradiated seeds showed a decrease of 6.5 in percentage of germination. Of 520 seeds irradiated 88.2 per cent germinated, while of 362 seeds used as controls 94.7 per cent germinated.

Soaked seeds exposed to medium doses (5-10 E.) showed, during the first three weeks, a checking of growth which was followed by a transitory acceleration of development. This temporarily increased growth was never sufficient to cause an increase over that of the controls in the total dry weight of mature plants from irradiated seeds or seedlings.

Different seeds of the same species may react variously to the same dosage of X-rays, as shown in one experiment where *Helianthus* plants grew eight times as tall as others from seeds irradiated similarly and exposed to identical environmental conditions. Experi-

ments with animals have shown that different individuals of the same species often exhibit considerable variation in their susceptibility to X-rays.

Fasciation is a fairly constant character produced by X-rays, as this occurs not only in irradiated sunflowers but in irradiated tomato and cosmos plants as well. By irradiating seeds and seedlings, fasciations of stems, leaves, and flowers were induced. In some cases these appeared as early as the fourth week of growth. The stems became flattened, showing bifurcations or splittings. These branches usually divided again. This branching of stems was very noticeable because the sunflowers used were of a non-branching type. Of the eighteen groups (consisting of eighty-one controls) which were grown to maturity, all were strictly one-stemmed individuals. Fasciations in leaves were shown by changes in number, position, size, and shape. Phyllotaxy was distorted and the number of leaves was commonly increased. Fasciation of the flower head was present in many forms: fusion in the involucrel region, forking of the stalk below the involucre with two distinct heads resulting, and the appearance of triradiate heads.

A frequent explanation for fasciations is that they are induced by changed internal conditions. Care was taken to subject controls, irradiated seeds, and irradiated seedlings to the same environmental conditions in the greenhouse. This could not insure absolutely equal internal conditions of nutrition, however, and it is possible that the plane of nutrition is much lower in the experimental material than in the controls.

It has been maintained (40, 41) that irradiations from either X-rays or radium cause irreversible changes in the protoplasm of the cell. This could hardly have occurred in the protoplasm of the sunflower cells, for, with light or medium doses, apparently normal growth takes place after recovery from the period of growth-checking.

Seeds in air-dried condition were found to be less easily influenced by the action of X-rays than were those with a water content above 50 per cent. Seedlings beginning active growth were more sensitive than resting or soaked seeds. These results are in accord with those of practically all other investigators in this field. Animal

cells also have been found by others (2, 3) to be more susceptible to X-rays during mitosis than during rest.

Catalase activity is lessened in young seedlings whose seeds have been exposed to medium doses of irradiation. With older seedlings which are beginning to show recovery from the checking effects of irradiation, the activity is almost equal to that of the control. With seedlings whose seeds were exposed to killing doses of X-rays, a greater depression of catalase activity was noted.

Depressed respiration accompanies the marked inhibition of growth caused by heavy irradiation. Soaked seeds given heavy dosage were placed on moist cotton in a respirometer which was supplied with CO_2 -free air. The amount of CO_2 liberated per unit of absolute dry weight was used as an index to respiratory activity. When compared with the CO_2 output of the control, that of seedlings from irradiated seeds showed a decided decrease.

Oxidase activity remains unchanged in seedlings grown from irradiated seeds. Checking of growth accompanying heavy irradiation must differ from that associated with dwarfing due to excessive watering, drought, disease, or some unknown agencies.

Histological study of the meristematic region of the tip of radicles from irradiated seeds showed marked differences in appearance from that of the controls. Cells from irradiated material showed elongation, great vacuolation or entire absence of protoplasm, and the absence of nuclei from many cells. The average size of the cells in the meristematic region of the radicle from irradiated material is twice the cross-section area of the control.

Increase of xylem with a corresponding decrease of pith cells is apparent in the hypocotyl region of mature plants whose seeds have been irradiated. The character of the elements differs in the two stems. In cross-sections from the hypocotyl regions of irradiated material, the pith cells are much smaller in diameter and are thicker walled than in control stems. The elements of the xylem in the former are much smaller in diameter, and arranged more compactly. Even in seedlings nine days old, a striking difference is shown in the amount and character of xylem. There is here also a much greater percentage of xylem in the irradiated specimens, with individual cells showing a similarity in size, rather than the wide diversity in

size of cells which is typical for xylem of young *Helianthus* stems. Epidermis on the lowest internodal regions of mature plants from seeds which had received medium doses of irradiation was noticeably dark brown in color, the cells irregular in outline, and the material when tested microchemically was found to be impregnated with considerable suberin. Tests on the epidermis of the control showed no suberin present.

Summary

1. Irradiation of soaked seeds of *Helianthus annuus* causes a slight decrease in percentage of germination. The average total growth of the plants is less, and the average time of blossoming is shortened, even with doses so light that there are no immediate visible effects. A checking effect, proportional to the doses, is evident during the first three weeks of growth after seeds have been irradiated with medium doses. On unfolding, the leaves present a peculiar pitted or mosaic appearance, which seems to be due to an unequal distribution of chlorophyll, when medium or heavy doses are given the seeds. Heavy dosage does not stop development of soaked seeds until the cotyledons have been partially or wholly pushed through the soil. The root tip becomes brown in color and no growing stem tip appears.

2. Fasciations of stems, leaves, and flowers are induced by irradiation of seeds and seedlings with medium doses. Stem fasciations commonly took the form of flattening and dichotomous splitting of the main stem, which showed the presence of many blossoming branches instead of a single flower head. Fasciation of the flower head was represented by fusion in the involucre region and by forking of the stalk below the involucre, with two or three distinct heads resulting. Many leaf abnormalities,—ligulate leaves, altered phyllotaxy, and incipient fasciation, which marked earlier periods of growth, often disappeared before maturity.

3. Catalase activity is depressed in young seedlings whose seeds have been exposed to medium doses of X-rays. With older seedlings which are beginning to show recovery, however, there is but slight reduction in activity noticed.

4. Depressed respiration is associated with stunted growth when this accompanies heavy irradiation. The amount of carbon

dioxide liberated from irradiated seedlings is less than that from controls similarly treated.

5. Normal oxidase activity is found in seedlings whose growth has been inhibited by heavy doses of X-rays.

6. Cells of the radicle tip from seedlings whose growth has been inhibited by heavy irradiation show elongation, great vacuolation or entire absence of protoplasm, and the absence of nuclei from many cells. The average size of the cells in the meristematic region of the radicle tip of irradiated material is greatly increased over that of the control. Xylem is increased at expense of the pith, and there is a greater suberin development in the hypocotyl regions of mature plants from irradiated seeds than in those of the control. The increase in vascular tissue in irradiated material is apparent even in seedlings but nine days old.

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POSSIBLE ETIOLOGICAL RÔLE OF PLASMODIOPHORA TABACI IN TOBACCO MOSAIC

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Introduction

The recent papers of ECKERSON² and JONES³ have again compelled consideration of the possible association of protozoa and mosaic of potato, tobacco, and tomato plants. Miss ECKERSON reports the discovery of actively moving flagellated and amoeboid bodies, as well as "spores" in the mesophyll cells of tomato plants affected with mosaic. JONES reports success in culturing a myceto-zoan (named by him *Plasmodiophora tabaci*) from tobacco tissues affected with mosaic-like symptoms, and the development of identical symptoms following the inoculation of healthy plants with such cultures. He also reports that he was able to find the plasmodial stage of the protozoan in tissues of tobacco plants with similar symptoms, and that healthy plants were entirely free from it.

The present investigation was undertaken to ascertain whether there is a constant association of JONES' organism with tobacco mosaic, and whether it plays any rôle in the etiology of the disease.⁴ The following lines of experiments have been carried out: (1) Tissues from healthy and diseased tobacco plants were cultured, to determine whether a correlation exists between the mycetozoan and mosaic. (2) Healthy tobacco plants were inoculated with cultures of *P. tabaci* obtained originally from healthy and diseased tissues, to ascertain whether such cultures carried the virus of tobacco mosaic.

¹ Joint contribution from the Department of Botany and the Department of Hygiene and Bacteriology of the University of Chicago.

² ECKERSON, SOPHIA H., An organism of tomato mosaic. *BOT. GAZ.* 81:204-209. 1926.

³ JONES, P. M., Structure and cultural history of a mycetozoan found in tobacco plants with mosaic-like symptoms. *BOT. GAZ.* 81:446-459. 1926.

⁴ As work of this character involves different techniques, it was thought best to carry out the experiments with the cooperation of several men of different training. Of the three authors, P. M. JONES is the discoverer of the mycetozoan, G. K. K. LINK is a plant pathologist, and W. H. TALIAFERRO is a protozoologist.

(3) Juice of diseased plants was filtered through diatomaceous and porcelain filters, the filtrate cultured, and also inoculated into young tobacco plants to ascertain whether *P. tabaci* can be cultured from known infective filtrates. (4) Cultures of *P. tabaci* were similarly filtered and the filtrates cultured to test the filterability of the mycetozoon. The infectivity of such filtrates was also tested by suitable inoculations.

The virus used in these experiments was obtained from tobacco plants kindly furnished us in November, 1925, by Dr. J. C. JOHNSON of the University of Wisconsin. In these experiments, two varieties of tobacco were used, Connecticut Broad Leaf and Kentucky Mammoth.

I. Culture of tissues

In carrying on the culture work the methods used by JONES (*loc. cit.*) were followed, with modifications as the work progressed. The cultures were made in Erlenmeyer flasks with necks large enough to permit passage of cover glasses, which were used in examinations for presence of the organism. At first Knop's solution was used, but later it was found that distilled water was equally efficacious. The flasks containing the medium and cover glasses were sterilized in an autoclave at 15 pounds pressure for 45 minutes. Young leaves were used in most of the cultures, but stems and roots were also employed. The tissues were so manipulated that there was no possible contamination from one lot to another during handling. Immediately after collection each leaf or part of leaf, stem, or root was rinsed in water, washed in a solution of mercuric chloride (1:1000) for 1-3 minutes, and then rinsed in sterile water. Lots DA, DB, and DC of diseased tissues, and lots HB, HC, and HF of healthy tissues were prepared in this manner. Later tissues were collected similarly but were not washed in mercuric chloride. These were lots DD, DE, and DF of diseased tissues, and HE and HF of healthy tissues.

The cultures were incubated at room temperatures (the range being 15°-25° C.); some in diffuse light and some in darkness. They were examined the first time after 15-20 days, and then at intervals of a week. For examination, a cover glass was removed with a flamed inoculation needle and then mounted face up on a slide. The results of these experiments are assembled in table I. No healthy plants

were cultured in the first lot. In samples DA 1-5 of January 30, the leaves were cut so that each culture contained only a portion of a leaf. Lots DA and DB of March 20, DC of May 15, DE of May 26, and DF of August 3 were run against the controls HB, HC, HE, and HF respectively. In these experiments only entire leaves were used, while the cultures of roots and stems consisted of tissue fragments.

TABLE I
CULTURES OF DISEASED AND HEALTHY TISSUES FOR PROTOZOA

CONDITIONS OF EXPERIMENT	PLANT	ORGAN	DATE OF MAKING CULTURE	DATE OF CLOSING CULTURE	NO. OF CULTURES	NO. OF CULTURES WITH ORGANISMS
Diseased tissues, washed in water, placed in mercuric chloride, and rinsed in sterile water	DA 1-5	Portion of leaf	January 30	February 19	16	9
	DA 5	Root	January 30	February 19	2	1
	DA 6-7	Stem	January 30	February 19	2	2
	DA 7	Petiole	January 30	February 19	1	1
	DA 1-5	Leaf	March 20	May 4	10	10
	DB 2,6,7	Leaf	March 20	May 24	9	9
	DB 1,6,6,9	Root	March 20	May 24	6	6
	DB 2,4,6,8	Stem	March 20	May 24	7	7
	DC 1-5	Leaf	May 15	June 17	25	13
					Total . . 78	58
Diseased tissues, washed in sterile water only	DD 1-3	Entire top of plant	May 17	May 27	3	3
	DE 1-10	Leaf	May 26	June 17	10	8
	DF 1-26	Leaf	August 3	August 26	26	21
					Total . . 39	32
Healthy tissues, washed in water, placed in mercuric chloride, rinsed in sterile water	HB 1-10	Leaf	March 20	May 4	10	1
	HC 1-25	Leaf	May 15	June 17	25	0
	HF 1-5	Leaf	August 3	August 26	5	3
					Total . . 40	4
Healthy tissues washed in sterile water only	HE 1-11	Leaf	May 26	June 17	11	10
	HF 1-5	Leaf	August 3	August 26	5	2
					Total . . 16	12

A consideration of the data in table I indicates that there is no significant difference between the results obtained when healthy and diseased tissues were cultured, provided the tissues were not washed (sterilized) in mercuric chloride. On the contrary, there was a marked difference between healthy and diseased lots provided the tissues were first washed (sterilized) in mercuric chloride. Thus, out of a total of 39 cultures from diseased plants not washed in mercuric chloride, 32 showed JONES' organism. Similarly, from 16 cultures

of healthy plants not washed in mercuric chloride 12 showed it. On the other hand, from a total of 78 individual cultures from diseased tissues which were washed (sterilized) in mercuric chloride 58 showed

TABLE II
INOCULATIONS WITH CULTURES FROM DISEASED PLANTS
FOLLOWED BY DEVELOPMENT OF MOSAIC

DATE OF INOCULATION	SOURCE OF CULTURE	PROTOZOAN IN CULTURE	INOCULATED PLANTS			CONTROLS		DATE OF CONCLUSION OF EXPERIMENT
			Total	Diseased	Healthy	Diseased	Healthy	
February 13	DA 4 Leaf...	None	3	2	1	0	3	March 1
	DA 4 Leaf...	Flagellates	3	3	0	0	3	
	DA 4 Leaf...	Cysts	3	3	0	0	3	
	DA 5 Root...	Flagellates	3	3	0	0	3	
	DA 5 Tip of plant.	Amoebae	3	3	0	0	3	
March 16	DA 1 Leaf...	Cysts, "parasites"	14	9	5	1	6	April 7
March 19	DA 1 Leaf...	Cysts, "parasites"	20	20	0	0	15	May 5
	DA 3 Leaf...	Flagellates	15	15	0	0	15	
April 7	DA 2 Leaf...	Cysts, "parasites"	6	6	0	0	4	May 5
	DB 1 Leaf...	Amoebae, flagellates	6	2	4	0	4	
	DB 6 Root...	No organisms	6	1	5	0	4	
	DB 6 Root...	Flagellates and ciliates	6	2	4	0	4	
May 5	DA 4 Leaf...	Cysts	5	1	4	0	4	June 2
	DB 7 Leaf...	No organisms	6	2	4	0	8	
	DB 7 Leaf...	Cysts, "parasites"	5	4	1	0	4	
	DB 7 Leaf...	Cysts	5	3	2	0	4	
	DB 7 Leaf...	Cysts, "parasites"	5	1	4	0	4	
	DB 8 Leaf...	Flagellates and ciliates	6	2	4	0	4	
June 6	DD 1 Top of plant.	Amoebae, flagellates, cysts	3	1	2	0	8	June 24
	DE 1 Leaf...	No organisms	3	1	2	0	8	
	DE 2 Leaf...	Flagellates	2	2	0	0	8	
	DE 3 Leaf...	Flagellates	2	1	1	0	8	
June 19	DE 5 Leaf...	None	6	6	0	0	2	July 17
	DE 6 Leaf...	None	6	6	0	0	2	
June 20	DE 4 Leaf...	None	5	1	4	0	4	July 17
	DE 5 Leaf...	None	5	4	1	0	4	
	DE 6 Leaf...	None	7	5	2	0	4	
Total.....			159	109	50	1	143	

organisms; but in 40 cultures from similarly treated healthy plants only 4 showed organisms.

II. Inoculation experiments

The inoculation experiments were made mainly with cultures reported in table I, and were designed primarily to ascertain whether the power to produce mosaic could be separated from the presence of *P. tabaci*, or whether the two were always associated. We were

greatly aided by the discovery, as already noted in the previous section, that typical cultures of *P. tabaci* could be obtained from healthy plants which had never shown evidences of the disease. As inoculations were made with cultures containing the organisms, both from diseased and healthy tissues, and with cultures from diseased tissues which did not contain the organisms at the time of inocula-

TABLE III
INOCULATIONS WITH CULTURES FROM DISEASED PLANTS
NOT FOLLOWED BY DEVELOPMENT OF MOSAIC

DATE OF INOCULATION	SOURCE OF CULTURE	PROTOZOAN IN CULTURE	INOCULATED PLANTS			CONTROLS		DATE OF CONCLUSION OF EXPERIMENT
			Total	Diseased	Healthy	Diseased	Healthy	
February 12	DA 5 Root...	None	3	0	3	0	3	March 1
May 5	DA 4 Leaf...	Amoebae, flagellates Cysts (cultures 10 days old)	4	0	4	0	4	June 2
	DA 4 Leaf...		5	0	5	0	4	
	DB 9 Stem (1 year old)...	Amoebae	3	0	3	0	4	
May 26	DD 1 Entire top...	Amoebae, cysts, ciliates, and ciliate cysts	4	0	4	0	10	June 24
	DD 2 Entire top...	Amoebae, flagellates	4	0	4	0	10	
	DD 3 Entire top...	Flagellates, cysts.....	4	0	4	0	10	
June 1	DE 1 Leaf....	Flagellates	2	0	2	0	8	June 24
June 19	DE 4 Leaf....	None	6	0	6	0	2	July 17
	DE 7 Leaf....	None	6	0	6	0	2	
June 20	DE 7 Leaf....	Amoebae, flagellates, cysts	5	0	5	0	4	July 17
July 13	DE 4 Leaf....	None	5	0	5	0	5	July 27
	DE 7 Leaf....	Amoebae, cysts	5	0	5	0	5	
		Total.....	56	0	56	0	71	

tion, it is essential to note these various factors in evaluating the experimental data given in tables II, III, and IV. Furthermore, in view of the fact that the stage of the organism might be a factor in infection, it is significant that it was possible to carry out inoculations with all stages of the life history of the organism reported by JONES save that of the plasmodium. Even here, however, some relevant data were obtained in filtration-inoculation experiments. All of the inoculations were made with mixed cultures save one set made on July 30. This was made with a pure culture of cysts and flagel-

lates in Knop's solution obtained from a single plasmodium which had been isolated and transferred with a capillary pipette. Inoculations were made by means of needles with a wrapping of cotton. In the later experiments each plant was inoculated with a separate sterilized needle. The needle was placed in the inoculum until the cotton had become thoroughly soaked, and the leaves were then punctured and scarified. The controls were punctured and scarified with sterile needles. These experiments are reported in tables II and III.

In table II are grouped the inoculation experiments in which cultures of diseased tissues were used, and in which the disease was

TABLE IV
INOCULATIONS WITH CULTURES FROM HEALTHY PLANTS

DATE OF INOCULATION	SOURCE OF CULTURE	PROTOZOAN IN CULTURE	INOCULATED PLANTS			CONTROLS		DATE OF CONCLUSION OF EXPERIMENT
			Total	Diseased	Healthy	Diseased	Healthy	
June 18	{HE 1	Amoebae, flagellates	6	0	6	0	4	July 17
	{HE 2	Flagellates, cysts	6	0	6	0	4	
June 19	{HE 1	Amoebae, flagellates	5	0	5	0	16	July 17
	{HE 2	Flagellates, cysts	5	0	5	0	16	
July 13	{HE 1	Gametes	5	0	5	0	5	July 27
	{HF 1	Amoebae, flagellates	4	0	4	0	5	
July 30	HE 3	Plasmodia, flagellates	10	0	10	0	10	August 14
		Total	41	0	41	0	60	

produced either in all or only some of the plants; while in table III are reported the experiments in which inoculation with cultures of the diseased tissues completely failed to produce the disease. Some of these cultures (tables II and III) did not show the organism at time of inoculation.

Out of the 214 control plants inoculated, only one developed mosaic; out of the 215 plants inoculated with cultures, 109 developed the disease; and of the 67 plants inoculated with cultures which at the time of inoculation did not show the organism (although they came from diseased plants), 28 developed mosaic.

In table IV are reported the results of inoculations with cultures obtained from healthy plants. *P. tabaci* was present in abundance

in these cultures. One of these (HE 3) was a pure culture of cysts and flagellates derived from a single plasmodium. Forty-one plants were inoculated and none developed the disease. Sixty controls were used and remained healthy.

On the supposition that this organism, if it were the causal agent of mosaic, might enter through the roots of plants, an experiment was conducted in which the cultures from diseased plants were poured on the soil of potted plants. In one lot the roots of the plant were cut, while in the other they were left intact. Out of 14 plants with cut roots, 12 developed the disease, while out of 43 plants with the roots intact, only one developed it. Twelve plants were used as controls and all remained healthy.

III. Filtration and inoculation experiments

In view of the enormous mass of evidence that the causative agent of mosaic passes through diatomaceous and porcelain filters, it seems reasonable to suppose that if *P. tabaci* is the causative agent it must have some filterable stage. In the experiments of this section infective tobacco juice was filtered, the filtrate again tested for infectivity, then allowed to stand for several weeks to see whether *P. tabaci* appeared; and finally, as *P. tabaci* did not develop in any of the filtrates, these were tested for their suitability as culture media by inoculation with a known strain of *P. tabaci*. A few filtration experiments were carried out also with cultures of *P. tabaci*. In these experiments the work was so divided that the grinding of diseased tissues for juice was done by one person, the filtration by another, and the inoculation of plants by the third. This was to avoid all possibility of transmission of the virus by the person carrying out inoculations. Some of the filtrates were filtered into sterile flasks containing sterilized leaf tissues which might provide a suitable food medium for the organism, in case it should pass through the filters, and others were filtered into flasks containing only cover glasses. In one of the experiments (14 J and 15 J) the juice of each diseased plant was filtered through a separate filter, but the filtrates were then divided into several flasks. In the filtration work, Mandler, Berkefeld, and Pasteur-Chamberland filter candles were used. In each case the entire apparatus was set up complete, wrapped in

paper, and autoclaved for 30 minutes at 15 pounds pressure. When the diatomaceous candles were used an agar seal was deposited around the bottom of the candle just before use, in order to avoid leakage. The Pasteur-Chamberland candles were so arranged that the material was sucked into the candle and passed through a section of pressure tubing into a flask. Before filtration the tissue extract or the cultures were heavily inoculated with *Bacillus prodigiosus*. The filtrates were tested for *B. prodigiosus* 12 hours after filtration and again (24 hours later) when part of the filtrate was removed with sterile pipettes for inoculation purposes. Relevant data in regard to filtration conditions, such as pressures, time, P_H , etc., are given in table V. In no case did *B. prodigiosus* pass through the two Pasteur-Chamberland filters that were used. About half of the diatomaceous (especially the Mandler) candles leaked *B. prodigiosus* and were therefore discarded. None of the experiments with faulty filters are included in the table.

The filtration experiments with tissue extracts can conveniently be considered in two lots. In the first lot (table V, 4-13) the filtrates were free from *B. prodigiosus* 24 hours after filtration. In the operation of removing material for inoculation, however, they were opened several times in a very dusty room, and all later became infected with common air contaminants. As many stages of *P. tabaci* feed upon bacteria, it was felt that such contaminated filtrates would be particularly conclusive if the mycetozoan did not develop in the filtrates. An examination of the data in table V shows that although these filtrates were highly efficacious in producing mosaic, *P. tabaci* developed in none of them. This is true although the filtrates contained what we consider suitable food for the mycetozoan, and in spite of the fact that later inoculations of the filtrates with *P. tabaci* showed conclusively that the mycetozoan could live and grow in such a medium.

In the second lot of filtrations (14 J and 15 J) the filtrates were opened in a dust-proof chamber, and hence showed less (5 out of 14) contamination from the air. The contaminated cultures were discarded. The data gained from this experiment (table V) again show that the filtrates were infective to tobacco plants, but in no case did *P. tabaci* appear in them. Here, again, later inoculation of the fil-

trates with *P. tabaci* showed that the mycetozoan could live and grow in them.

The third series of filtrations, inoculations, and cultures was carried out with cultures of *P. tabaci* (table V). In filtrations 4 C

TABLE V
FILTRATION, INOCULATION, AND CULTURE EXPERIMENTS
WITH JUICES OF DISEASED PLANTS, AND
CULTURES OF *P. TABACI*

MATERIAL*	FILTER*	PRESSURE IN CM. OF HG.	TIME IN MINUTES	P _H OF SOLUTION	INOCULATIONS OF PLANTS						EXAMINATION OF FILTRATE		EXAMINATION OF FILTRATE 7 DAYS AFTER ADDITION OF CULTURE OF MYCETOZOAN
					Date	Date of close of observations	Controls		With filtrates		July 30	August 7	
							Total	Diseased	Total	Diseased			
4-C.	B	63	25	8.0	7/10	7/17	5	0	7	0	0	0	Amoebae, cysts; Bac.
5-J.	B	63	20	6.6	7/10	7/17	5	0	7	7	Bac.	Bac.	Bacteria
7-J.	M	67	120	6.4	7/12	7/19	5	0	7	5	Bac.	Bac.; cysts	Cysts; Bac.
9-C.	B	65	30	8.0	7/14	8/7	5	0	7	0	Bac.	Bac.	Amoebae, cysts; Bac.
11-J.	P	65	20	6.4	7/16	7/23	5	0	4	4	Bac.	Bac.	Flagellates; Bac.
12-C.	B	70	15	6.6	7/16	7/22	4	0	4	4	Bac.	Culture lost	Culture lost
13-J.	P	65	20	6.4	7/16	7/22	4	0	4	4	Bac.	Culture lost	Culture lost
14-J.	P	64	25	6.4	8/3	8/20	10	0	10	10		August 14
a.	...										Bac.		Culture discarded August 14
b.	...										Bac.		Culture discarded August 14
c.	...										Fungus		Flagellates; Bac.
d.	...										o		Flagellates; Bac.
e.	...										o		Flagellates; Bac.
f.	...										o		Flagellates; Bac.
g.	...										o		Flagellates, cysts; Bac.
15-J.	P	64	25	6.4	8/3	8/20	10	0	10	10		August 14
a.	...										Bac.		Culture discarded August 14
b.	...										Bac.		No stage of mycetozoan; Bac.
c.	...										o		No stage of mycetozoan; Bac.
d.	...										o		Cysts; Bac.
e.	...										o		Flagellates, cysts; Bac.
f.	...										o		Flagellates, cysts; Bac.
g.	...										o		Flagellates, cysts; Bac.

* C, culture of mycetozoan from diseased plants; J, juice extracted from diseased plants; 5 J, 7 J, 11 J, and 13 J, separate lots of juice; 14 J a-g and 15 J a-g, portions of two separate lots of juice; B, Berkefeld filter; M, Mandrel filter; P, Pasteur-Chamberland filter.

and 9 C cultures containing plasmodia were used. The filtrates of these cultures did not develop the organism, although subsequent inoculations with a culture of *P. tabaci* proved that the filtrates were a suitable medium for growth of the mycetozoan. The filtrates were

not infectious. Unfortunately, however, it developed later that we do not have, as was thought when these cultures were filtered, any data relative to the infectivity of the cultures before filtration; consequently these experiments merely prove that the plasmodial stage of the organism did not pass the filters. 12 C was a culture from a diseased plant (DE 5, tables I and II). This culture had proved consistently infective during months when inoculated into plants. It never developed recognizable stages of the mycetozoan, however, but at one time it contained bodies which JONES thought might be a gametic stage. The filtrate was infectious and the organisms did not develop in the filtrate, although it proved to be a suitable medium when inoculated with a culture of *P. tabaci*. These data again indicate that the stages of the organism tested were not filterable.

Discussion

The culture experiments listed under I, although inconclusive in regard to the possible etiological rôle of *P. tabaci* in mosaic disease, bring out several facts of importance in regard to the distribution of the mycetozoan. They conclusively demonstrate that some stage of the organism, probably the cysts, are on the surface of many healthy and diseased plants. After a partial sterilization of the surface of the plants, the organism was found much more frequently in (or on) diseased than healthy plants. It would seem that this may be explained in one of three ways:

1. Diseased plants may present a rougher surface, and hence resistant stages of *P. tabaci* could more easily escape contact with mercuric chloride than in the case of healthy plants. If this possibility be the true one, *P. tabaci* would be considered only as an accidental contaminant of the plant's surface.

2. Diseased plants may present more breaks in the epidermis than healthy ones. It is possible that *P. tabaci* penetrates such breaks and lives within the parenchyma. If this be true, *P. tabaci* would probably be a secondary invader without any significant part in the production of this disease. Such an explanation has much in its favor, as it agrees with the rest of the experiments which indicate that the mycetozoan is not the causative agent, and it agrees with JONES' (*loc. cit.*) contention that the plasmodium of *P.*

tabaci can be found in the tissues of the leaf. It also is not out of harmony with the fact that some healthy leaves, even after washing in mercuric chloride, show the organism, as these may have been slightly damaged in other ways than by mosaic disease.

3. There is the possibility that *P. tabaci* is a frequent parasite of tobacco plants, and that it is the primary invader which later allows the entrance of the virus of tobacco mosaic. At present there seems little to commend this possibility.

The inoculation experiments listed under II indicate beyond reasonable doubt that, for the stages tested, *P. tabaci* is not the causative agent of tobacco mosaic, because cultures of the protozoan were effective in producing mosaic only when originally obtained from diseased plants, never when obtained from healthy ones. Furthermore, some cultures made from diseased plants and never showing the organism produced the disease. These results are just what would be expected if the disease is produced by an unknown virus, and *P. tabaci* is a common contaminant of many plants.

The filtration and inoculation experiments listed under III indicate further that *P. tabaci* is not the causal agent of mosaic. All the filtered juices from diseased plants produced the disease promptly, but none developed the organism in culture, in spite of the fact that later inoculation of the filtered juice with *P. tabaci* showed that it was a suitable medium for the growth of the mycetozoon. Similar filtration and cultural experiments with cultures of the plasmodial stage organism proved that it does not pass the filters used in the stage which JONES reported as occurring in diseased tissues.

Conclusions

1. *Plasmodiophora tabaci* can be cultivated from both healthy plants and those affected with mosaic, provided they are not washed in an antiseptic solution such as mercuric chloride. If they are washed in mercuric chloride *P. tabaci* can frequently be obtained from diseased but only rarely from healthy plants.

2. Inoculation of tobacco plants with cultures containing various stages of *P. tabaci* is followed by mosaic only when the cultures are derived from diseased plants, in which case a concomitant mosaic virus could be present and carried by dilution.

3. Cultures prepared from diseased leaves are sometimes negative for *P. tabaci* and still efficacious in the production of mosaic.

4. Filtrates from diseased plants are infective, but do not show *P. tabaci* after standing various lengths of time. Furthermore, such filtrates are suitable culture media for *P. tabaci*, as was demonstrated at the end of each experiment.

5. *P. tabaci* in the plasmodial stage does not pass the filters.

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TEMPERATURE AND RESPIRATION OF RIPENING BANANAS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 361

ALBERT JACKSON OLNEY

(WITH ONE FIGURE)

Introduction

The appearance of the banana as an important fruit on the American market is relatively recent, although it has been cultivated and used from earliest times. The first shipment to the United States (25) was made in 1804, and another in 1830, but it was not until 1866 that bananas were imported regularly, and then only in modest quantities to seaboard towns. During the last twenty-five years the banana trade has been extended throughout the United States until now it represents an important industry.

Bananas must be picked while still green, in order to withstand the long journey from the tropical countries to the markets. A week to ten days are required for transportation to points in the United States. If ripening begins in transit the fruit deteriorates before it reaches its destination, and results in heavy losses. The problem of chief concern, therefore, is that of finding improved methods of retarding the ripening in transit and storage so that the fruit may reach the market in a condition of high quality and attractive appearance. The degree of maturity when harvested is determined by the growers in a purely empirical way, the degree of maturity when picked depending on the distance to be shipped. Those going to Europe are harvested somewhat greener than those for the United States, and the greener lots are said to be poorer in quality than those picked when more mature (13). Shipments are made in specially constructed steamships providing reduced temperatures by ventilation or refrigeration. Naturally ventilated steamships are commonly used for shipments from Central America or Jamaica to the Gulf ports. Distribution is then made to the central portions of the country in refrigerator cars. Refrigeration shipments are held as near 16° C. as possible.

After arriving at their destination the bunches are hung in curing rooms at 17° – 20° C., providing 82–86 per cent humidity. The trade considers 15° C. the lowest safe temperature for green bananas, and 14° C. as the minimum for ripe ones. Chilling is said to occur at lower temperatures, injuring the quality and preventing the development of attractive appearance. Cargoes sometimes arrive at the ports too ripe for general distribution, while other shipments apparently handled in the same manner arrive in good condition. The causes for such occurrences are not well understood by importers. There is a general belief that a few ripe or ripening bananas among a lot of green ones greatly accelerate the ripening of the cargo.

The first noticeable development of the green fruit is the gradual change of color, accompanied by a softening of the pulp, due to the hydrolysis of pectose to pectin (17). The change from green to yellow takes place in 3–5 days at curing temperatures after arriving at their destination. Two or three days after the bright yellow stage, brown specks develop through the yellow, and the pulp reaches its highest flavor, aroma, and quality. This stage is short; the pulp soon becomes too soft, loses flavor, and begins to decay. When green the peel is filled with a white viscous fluid and the pulp is acid, bitter, and astringent. GERBER (14) cites BOUSSINGAULT and CORENWINDER (9, 10) as attributing the acidity of bananas to malic acid, but he was able to show only the presence of citric acid. As the ripening continues the peel becomes dry and leathery, and the pulp increases in sweetness until the fully ripe stage is reached.

A number of investigators have studied various phases of the ripening process of bananas. The Jamaica Department of Agriculture (27) investigated the gases given off by oranges and bananas. The carbon dioxide liberated was thought to retard the ripening, but unknown gases or emanations which they thought were given off by the oranges were believed to induce premature ripening. The practical deduction was drawn that separate storage was desirable for the two sorts of fruit during sea transportation. The chemical changes taking place in ripening bananas have been reported by GORE (16), MARCANO and MUNTZ (21), RICCIARDI (24), DOHERTY (12), COLBY (8), BALLAND (4), ATWATER and BRYANT (1), CHACE, TOLMAN and MUNSON (7), PRINSEN GEERLIGS (22), YOSHIMURA

(28), REICH (23), TALLARICO (26), and BAILEY (23). Most of these analyses were made on green and ripe fruit. GORE (16) makes a sweeping criticism of the early analyses because their results were invariably expressed in terms of the percentage of the pulp when analyzed, so that the data are on a constantly shifting basis, since the peel continually loses weight while the pulp increases in weight during ripening.

LANGWORTHY and MILNER (20) studied the ripening of bananas in a respiratory calorimeter. They found that the volume of oxygen consumed was practically equal to the volume of carbon dioxide liberated. Their determinations show from the thermal quotient and carbon dioxide produced that the respiration was due entirely to the combustion of carbohydrates.

Materials and methods

The bananas used for the following experiments were obtained from the United Fruit Company through the kindness of Mr. J. W. LEATHERS, to whom thanks are gratefully accorded. Green bananas were obtained as soon as possible after arrival in Chicago. Two lots were received from Honduras, one ventilated and one refrigerated, and a ventilated and a refrigerated lot from Jamaica.

JOHNSTONE'S modification of Magness' respiration apparatus (19) was used to determine the amount of oxygen consumed and carbon dioxide liberated during the ripening. The apparatus was maintained at 20° C. in a Freas water bath. One hour was allowed for adjustments of temperature of the apparatus to that of the bath at the beginning of each experiment, and one-half hour between the daily runs when fresh quantities of NaOH were inserted for absorption of carbon dioxide. A Beckman thermometer was used to check the uniformity of the temperature of the bath. The temperature variation ranged from 0.05° to 0.20° C. The oxygen absorbed by the bananas was considered equal to the volume of water drawn into the cylinder. The amount of carbon dioxide liberated by the bananas was determined by titrating the NaOH, using the double indicator method (5). The individual bananas were cut apart and weighed before being put into the respiratory chamber. Later weighings were made at the end of each day's run. Four refrigerators operated at

2°, 7°, 12° and 17° C. were used for determining the effect of low temperatures on ripening.

Results

The results obtained from respiration studies are given in tables I, II, III, and IV. At the end of each experiment evidences of initial decay were usually apparent, although the pulp was in good condition. The maturity of the different lots was not uniform when re-

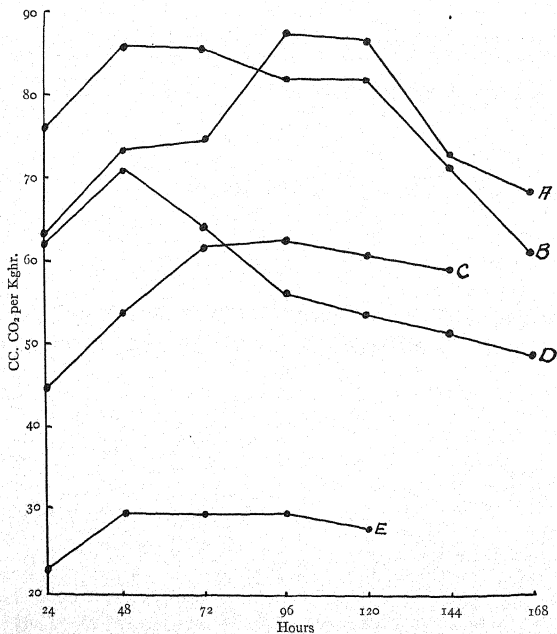


FIG. 1.—A, Honduras refrigerated 20°; B, Jamaica refrigerated 20°; C, Jamaica ventilated 20°; D, Honduras ventilated 20°; E, Jamaica refrigerated 12° C.

ceived, even when account is made for the method of cooling in shipment. The Jamaica ventilated lot appeared most mature, while the Jamaica refrigerated and the Honduras ventilated were similar in apparent maturity, and the Honduras refrigerated was greener than the others and not as ripe at the end of the experiment. Table V shows the daily loss in weight and production of carbon dioxide at 12° C. Fig. 1 indicates the nature of the respiration of the different lots at 20° C. and at 12° C. Table VI shows the effects of low temperatures on ripening.

The Jamaica ventilated bananas (table I) ripened very rapidly, and by the end of six days were becoming soft, showing initial decay,

TABLE I
JAMAICA VENTILATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PER- CENT- AGE LOSS IN WEIGHT	KGMHR	CALOR- IES PER KGMHR	CC. CO ₂ PER KGMHR	CC. O ₂ PER KGMHR	CO ₂ /O ₂ PER KGMHR	REMARKS
March 14, 23 hours..	325.0	0.06	7.475	0.22	44.94	44.151	1.01	Slightly yellow
March 15, 25 hours..	324.8	0.18	8.120	0.27	53.71	49.261	1.09	Mostly yellow
March 16, 23.5 hours	324.2	0.27	7.618	0.31	61.57	55.131	1.11	Yellow
March 17, 23.5 hours	323.3	0.06	7.597	0.32	62.97	53.311	1.18	Yellow
March 18, 23.5 hours	323.1	0.18	7.593	0.31	60.50	50.041	1.20	Brown
March 19, 23.5 hours	322.5	0.28	7.578	0.30	58.80	46.221	1.24	Very ripe

and were too ripe for handling on the market; however, the pulp was sound and had a rich aromatic flavor. The fruit inclosed in the respiratory chamber seemed to decay more rapidly than samples of the same lot stored in the laboratory, due to higher humidity. The loss in weight during this experiment was very low, about 60 per cent being due to liberation of carbon dioxide. The kilogram hours were determined by multiplying the kilograms of bananas by the number of hours in the run. The calories of heat produced were calculated from LANGWORTHY and MILNER'S (20) determinations, showing that 2.58 calories of heat are produced for each gram of carbon dioxide liberated. The volume of carbon dioxide given off and oxygen consumed were nearly equal in the daily runs, the respiratory quotient gradually increasing from 1.01 for the first run

to 1.24 at the end. There was a gradual increase in the volume of carbon dioxide produced until the bananas were fully yellow, followed by a slight decline.

The Jamaica refrigerated lot appeared green when received but ripened rapidly. The daily loss in weight was fairly uniform, and averaged about 0.4 per cent of the weight of the fruit per day, of

TABLE II

JAMAICA REFRIGERATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	KGMHR	CALORIES PER KGMHR	CC. CO ₂ PER KGMHR	CC. O ₂ PER KGMHR	CO ₂ /O ₂ PER KGMHR	REMARKS
March 28, 24 hours..	770.1	0.53	18.48	0.38	75.98	77.65	0.97	Green
March 29, 23 hours..	766.0	0.45	17.61	0.44	86.42	83.19	1.03	Yellowing
March 30, 23.5 hours	762.5	0.43	17.90	0.43	85.13	76.81	1.10	Mostly yellow
March 31, 23 hours..	759.2	0.41	18.20	0.42	82.47	76.37	1.07	Yellow
April 1, 24 hours....	756.1	0.41	18.40	0.42	83.72	74.97	1.11	Yellow
April 2, 23 hours....	752.9	0.42	17.30	0.36	70.48	60.11	1.17	Brown
April 3, 23 hours....	750.3	0.34	17.20	0.30	60.00	59.30	1.01	Very ripe

TABLE III

HONDURAS VENTILATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	KGMHR	CALORIES PER KGMHR	CC. CO ₂ PER KGMHR	CC. O ₂ PER KGMHR	CO ₂ /O ₂ PER KGMHR	REMARKS
April 11, 23 hours...	688.4	0.40	15.83	0.32	63.21	64.10	0.99	Light green
April 12, 23 hours...	685.6	0.42	15.76	0.36	71.34	62.78	1.13	Yellowing
April 13, 23 hours...	682.7	0.38	15.70	0.33	64.45	62.41	1.08	Mostly yellow
April 14, 23 hours...	680.1	0.35	15.64	0.29	56.52	53.06	1.06	Yellow
April 15, 23 hours...	677.7	0.37	15.58	0.27	54.27	49.08	1.10	Yellow
April 16, 23 hours...	675.2	0.34	15.53	0.26	52.48	49.58	1.05	Browning
April 17, 23 hours...	672.9	0.33	15.47	0.25	48.82	47.86	1.02	Very ripe

which 31 per cent was due to loss of carbon dioxide. The volume of carbon dioxide produced was considerably greater per kilogram per hour than that produced by the Jamaica ventilated lot. The greatest production of carbon dioxide occurred on the second day, followed by a gradual decline to the end of the yellow stage, and then a rapid falling off. The respiratory quotient was slightly more than unity except for the first day.

The Honduras ventilated bananas ripened at approximately the same rate as the Jamaica refrigerated, and the daily loss in weight was similar in amount. The largest production of carbon dioxide occurred on the second day and gradually declined to the end of the experiment. The respiratory quotient was slightly above unity in most cases.

TABLE IV
HONDURAS REFRIGERATED; RESPIRATION AT 20° C.

DATE AND TIME OF RUN	WEIGHT (GM.)	PERCENTAGE LOSS IN WEIGHT	KGMHR	CALORIES PER KGMHR	CC. CO ₂ PER KGMHR	CC. O ₂ PER KGMHR	CO ₂ /O ₂ PER KGMHR	REMARKS
April 18, 23.5 hours.	154.5	0.50	3.630	0.32	62.80	63.35	0.99	Green
April 19, 23.5 hours.	153.7	0.50	3.612	0.37	72.65	66.44	1.08	Slightly yellow
April 20, 23.5 hours.	152.9	0.39	3.593	0.38	74.59	73.75	1.01	Yellowing
April 21, 23.5 hours.	152.3	0.46	3.579	0.44	87.79	89.41	0.99	Yellow
April 22, 23.5 hours.	151.6	0.39	3.562	0.43	85.29	84.22	1.01	Yellow
April 23, 23.5 hours.	151.0	0.39	3.548	0.37	72.71	66.38	1.09	Slightly brown
April 24, 23.5 hours.	150.4	0.33	3.534	0.34	67.68	67.91	0.99	Ripe

TABLE V
JAMAICA REFRIGERATED; RESPIRATION AT 12° C.

DATE AND TIME OF RUN	WEIGHT	PERCENTAGE LOSS IN WEIGHT	KGMHR	CC. CO ₂ PER KGMHR	REMARKS
March 28, 24 hours.....	585.0	0.29	14.04	22.27	Green
March 29, 23 hours.....	583.3	0.25	13.42	29.96	No change
March 30, 22 hours.....	581.8	0.22	13.80	29.13	No change
March 31, 22 hours.....	580.5	0.24	12.77	29.73	No change
April 1, 23 hours.....	579.0	0.34	13.31	26.85	Slight change

The Honduras refrigerated bananas were less mature when received than any of the other lots. The loss in weight for the first day was 0.5 per cent of the weight of the fruit, and declined to 0.3 per cent for the last day of the experiment. About 25 per cent of this loss was due to liberation of carbon dioxide and the remainder to transpiration. The volume of carbon dioxide was relatively high per kilogram per hour, and higher than the Honduras ventilated, the maximum occurring on the fourth day. The respiratory quotient was more nearly unity than any of the other lots.

The respiration of the Jamaica refrigerated lot at 12° C. is shown in table V. The daily loss in weight was reduced to nearly one-half and the volume of carbon dioxide produced to about one-third that at 20° C.

The effect of low temperatures on the ripening of bananas is shown in table VI. The lots held at 2°, 7°, and a part of those at 12° C. were transferred April 5 to the 17° refrigerator, and the remainder of those held at 12° were continued at that temperature. The lot held at 17° ripened in six days, while those held at the lower

TABLE VI

EFFECT OF LOW TEMPERATURE ON RIPENING (PUT IN REFRIGERATORS MARCH 28)

DATE OF EXAMINATION	2° C	7° C	12° C	17° C
March 30...	Green	Green	Green	Quite yellow
April 1.....	Slightly darker	No change	No change	Yellow
April 3.....	Same	Same	Same	Ripe (removed)
April 5.....	Same	Same	Slightly yellow	Part put at 17° and part continued at 12°
April 7.....	Put at 17° Slight	Put at 17° Slight	Little change	
April 7-14..	Gradual change to a dull yellowish brown with black and dark brown areas; no bright yellow stage; pulp became soft, poor quality, lacking aroma; no marked differences in the three lots			Gradual change to yellowish green with black areas; remained hard; not eatable

temperatures showed little change during this time, and when transferred to ripening temperature failed to ripen normally. After eleven days they had developed a dull unattractive appearance and lacked quality and aroma. Those held continuously at 12° C. were still hard and green at the end of the experiment, although they were beginning to show signs of decay.

Discussion

The changes taking place during ripening of bananas are rapid, and the fully ripe period is very short, usually lasting about two or three days at ordinary temperatures. The rate of respiration at 20° C. was found to increase rapidly during the first two or three days and then gradually declined. The refrigerated lots, tables II and IV,

gave a high respiration rate followed by a rather sharp decline; while the ventilated lots, tables I and III, did not respire at so high a rate and declined more gradually. This difference in respiratory activity may have been due to an accumulation of sugars at the low temperatures, so that a more rapid respiration occurred when brought into ripening temperatures. The volumes of carbon dioxide liberated and oxygen absorbed were practically equal, as shown in the tables. In the combustion of carbohydrates the volume of carbon dioxide produced is exactly the same as that of oxygen consumed; that is, the respiratory quotient is 1.00. It appears therefore that the oxidation of carbohydrates is the principal process involved. This is also confirmed by inspection of analyses of the banana (16), which show that carbohydrates and water comprise about 96 per cent of the whole fruit, with less than 1 per cent of fats or proteins. Further to the point, LANGWORTHY and MILNER determined that the thermal quotient for normal ripening bananas indicates that the carbon dioxide produced is due solely to the complete combustion of carbohydrates. They found that 2.58 calories of heat are produced for each gram of carbon dioxide eliminated. Calculations on this basis show that approximately 0.3 calorie is produced per kilogram per hour during ripening at 20° C. This means the production of 10-15 calories of heat per hour from a single bunch of bananas at maximum respiration, and shows the possibility of a considerable rise in temperature in the ship or car during transportation. This may be an important factor in causing the occurrence of occasional shipments which become over-ripe in transit. It is important, therefore, not only that the surrounding temperature should be carefully regulated, but that proper ventilation should be provided to carry away the heat formed by the bananas themselves. It has been suggested that the heat liberated by the bananas during ripening may be due in part to bacterial action, but bacteriological studies by BAILEY (3) indicate that the inner part of the pulp is practically sterile, while the inner portion of the peel is sparsely inhabited by bacteria which he found were practically inactive until the fruit became ripe.

The loss in weight during ripening was relatively low, due to the highly cutinized surface of the peel and the high humidity maintained throughout the experiments. About one-fourth of the loss

was due to escape of carbon dioxide, and the remainder to transpiration of water. During ripening the pulp increases considerably in water content, due partly to absorption of water from the peel because of increased osmotic pressure as starch is changed to sugar, and partly from the combustion of glucose according to the equation $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$. The amount of carbon dioxide liberated at 12° was reduced approximately to one-third that at 20° C. The volume of oxygen absorbed at this temperature was not determined, and consequently the respiratory quotient cannot be shown, but the efficacy of reduced temperatures in retarding ripening is clearly indicated.

On March 28, samples of green bananas were put in refrigerators at 2° , 7° , 12° , and 17° C. Those held at 17° were fully ripened in six days, while those held at the lower temperatures were still very green. After eight days those held at 2° and 7° , and a part of those at 12° were put in the 17° refrigerator and held there for eleven days. It was soon strikingly apparent that the pre-refrigeration at 2° , 7° , and 12° had made a decided change in the character of ripening when placed at 17° C. All lots ripened slowly. The change in color was very gradual and the bright clear yellow failed to develop. At the end of the experiment the appearance of all lots was dull and unattractive, yellowish brown in color, spotted with blackened areas, and showing evidences of initial decay. The pulp had softened and appeared to be in good condition. The quality was almost as good as some often obtained on the market, but was noticeably flat and decidedly lacking in the aroma so characteristic of properly ripened bananas. These results are in agreement with the earlier work of GERBER (14). This indicates that a temperature of 12° or lower not only retards ripening but permanently affects the fruit, so that normal ripening does not occur when brought into ripening temperatures.

Summary

1. At 20° C. green bananas ripen rapidly, becoming fully ripe in 5-7 days after arrival in Chicago.
2. The respiratory quotient is approximately unity, indicating that the oxidation consists solely of the combustion of carbohydrates.

3. The rate of respiration increases rapidly at the beginning of ripening and falls off gradually.

4. Refrigerated bananas had a higher rate of respiration than those naturally ventilated, and declined more rapidly as they became ripe.

5. Respiration at 12° was reduced to about one-third that at 20° C.

6. The daily loss in weight was relatively low under the conditions of these experiments.

7. Approximately 0.3 calorie of heat is produced per kilogram per hour by ripening bananas at 20° C.

8. Refrigeration at 12° C. or lower not only retarded ripening in storage, but permanently prevented normal ripening when transferred later to a ripening temperature. The low temperatures prevented the development of bright yellow color, high quality, and aroma.

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STRUCTURE OF PENNSYLVANIAN PLANTS FROM ILLINOIS. I

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 362

J. HOBART HOSKINS

(WITH PLATES XXIII, XXIV)

Through the courtesy and interest of Mr. JULIUS HEGLER, of the Hegler Zinc Company, Danville, Illinois, the model coal mine of that company has been open for collecting, with the result that a number of coal balls have been obtained by Professor A. C. NOÉ. Some of these petrifications were kindly turned over to the writer, and a series of sections from one of these coal balls constitutes the material basis of this paper.

The Hegler coal mine produces coal from the Danville vein, known locally as no. 7 coal, and charted in that formation of the Pennsylvanian period known generally in Pennsylvania as the Conemaugh, and in Illinois as the McLeansboro. Unpublished investigations of this coal seam by paleobotanists and stratigraphers indicate that this vein may ultimately be given a higher position than that by which it is now known.

The Danville vein at the Hegler mine has a thickness of about 5 ft. 4 in. Below the coal is the usual fire clay, while above is a 7 ft. layer of black shale with flakes of pyrite, in which occur numerous concretions. The petrifications occur in groups or pockets in the upper 2 ft. of the coal. They are calcified, with pyrite occurring in varying amounts.

From one of these coal balls was obtained a series of sections which gives the structure of the ultimate rachis of a fernlike frond with pinnules bearing fructifications. The pinnule is of the *Pecopteris* type. The midvein is prominent and extends almost to the tip of the pinnule. The lateral veins, usually seven to nine on either side of the midvein, are given off at a wide angle and are simple. The pinnules are 6-7 mm. long and about 3.3 mm. in width, and are furled so that their edges approach the lower side of the midvein, forming a semi-chamber within which the fructifications are attached.

Sporangia occur in small groups or sori. These are arranged in a single series on each side of the median vein on the lower side of the pinnule. There is not a wide variation in the number of sporangia found in the different sori. With the exception of one group of six sporangia, the number in each sorus is either four or five. Groups of four are about twice as numerous as groups of five. Adjacent sori may vary in number.

The sporangia are found only in the mature condition with dehiscence either already completed, or with the spores completely separated apparently ready to be dispersed. The sporangia of each sorus are but slightly connected laterally at their bases, and are attached to a common pedicel to the lower surface of the pinnule. The sporangia in cross-section are roughly cylindrical, with the adjacent sides slightly flattened. The sporangia swell slightly below their points of attachment, so that their maximum diameters occur a little above their bases. The outline of the entire sorus in cross-section is somewhat star-shaped, with extremely obtuse points. In longitudinal section the sporangial cavity is ovoid in shape, with the longer axis at right angles to the pinnule. The distal ends of the sporangia are considerably prolonged beyond the sporangial cavity, to form bluntly acute apices by the elongation of the sporangial wall cells of this region on the sporangium.

The sporangia average 0.28 mm. in diameter and about 0.70 mm. in length. Spores are commonly found in large numbers within the sporangia, and average 0.017 mm. in diameter. Neither marking of the spore coat nor spore content can definitely be distinguished. Dehiscence occurred through a vertical cleft extending from near the point of attachment of the sporangia to near the free end, on the inner face of the sporangia.

The sporangial wall varies greatly in thickness in different parts of the sporangium. In most sections it appears to be but one cell in thickness, but in favorable sections there are good indications of two additional layers of cells closely applied to the outer prominent wall cells. These inner layers, where they occur, consist of small thin walled cells, elongated parallel to the long axis of the sporangia.

The cells of the outermost sporangial wall layer differ considerably in size. Those on the outer free surface in cross-section are

approximately square, the outer tangential wall being slightly longer than the inner wall, and comparatively large with exceedingly thick cell walls. Many of the cells have a dark content. A longitudinal section through this surface shows that these cells have a length about twice their width, and that the outline of their cell walls gives a reticulated appearance.

The cells of the adjacent walls are much smaller and have their cell walls less thickened. The smallest cells are at the very innermost part of the sporangia, where the cleft for dehiscence occurs. In cross-section the cells of the laterally adjacent walls of the sporangia are rectangular, the radial width being about half the tangential width.

The maximum elongation of the sporangial wall cells is seen at the distal end, where they form a continuation of the sporangium into a bluntly acute apex for a considerable distance beyond the sporangial cavity. The distal ends usually are slightly concrescent toward a common center. Due partially to the fact that the extreme distal end is slightly curved, and partially to loss before petrification, longitudinal sections usually fail to show the extreme tip of the sporangium. These sections then give the appearance of a sporangium with a rather rounded free end, capped by a group of larger heavy walled cells which seem to extend for a distance up the free side toward the base of the sporangium. In reality, however, the apices of the sporangia are not rounded, but more or less acute, the free end being prolonged beyond the sporangial cavity.

The pinnules to which the sporangia are attached have in transverse section one or two rows of larger heavy walled cells beneath the upper epidermis, which in almost every instance is not preserved. Below is a region of thin walled cells more or less compactly arranged and rather circular in section. In some sections there seems to be a slight development of palisade tissue beneath the heavy walled tissue. No stomata have been identified positively.

The midvein of the pinnule consists of a small circular group of tracheids accompanied by a few non-lignified elements. There is not the U-shaped bundle which is characteristic of the ultimate rachis. The sporangia are supplied by lateral veins, a few spirally thickened elements deploying and ending in the upper portion of the pedicels which attach the sori to the pinnules.

The ultimate rachis bearing the pinnules has a vascular system in the form of a wide U. The xylem cells are small in caliber and rather uniform in size, although those at the free ends of the U are slightly smaller and in longitudinal section are found to be spirally thickened in place of scalariform, as are the others. Fig. 7 shows the vascular connection of the ultimate rachis and pinnule. A small group of tracheids is separated from a free point of the bundle of the rachis and almost immediately turns sharply outward into the pinnule forming the midvein, to which a small number of lateral veins are connected.

Surrounding the xylem of the bundle are three or four rows of thin walled cells, of about the same caliber as the xylem, and bounded externally by a very narrow region (scarcely a row, although certainly appearing so in some places) of very small and thin walled cells. In the section figured it is not demonstrated that these very small and thin walled cells completely encircle the tracheids, as they are not identified immediately within the U. Most transverse sections of the ultimate rachis, however, give the appearance of having this tissue entirely surrounding the vascular bundle.

Many of the large cells beneath the vascular bundle of the ultimate rachis show a dark content and were probably mucilage or tannin cells. A band of sclerenchyma extends from between the points of the U-shaped bundle to the epidermis between the places of attachment of the pinnules. Multicellular hairs are sparingly present on the lower side of the rachis and pinnules.

The following summary of the sporangial characteristics of this specimen may facilitate comparisons with other fernlike fructifications: Sori composed of four or five, rarely six sporangia, and attached by short pedicels to the lower surface of pinnules of the *Pecopteris* type in a single series on either side of the midvein. Pinnules about 6-7 mm. long and 3.3 mm. in width. Sporangia ellipsoidal, roughly circular in transverse section with adjacent sides slightly flattened, and attached laterally at their very bases. Distal ends extended into slightly concrescent bluntly acute apices by the elongation of the sporangial wall cells. Sporangia about 0.28 mm. in diameter and 0.7 mm. in length. Wall cells on free surface of the sporangium much larger and with heavier walls than those on

the adjacent walls. Dehiscence occurs through vertical cleft on the innermost wall of the sporangia. Spores numerous, averaging 0.017 mm. in diameter.

In his classification of fernlike fructifications, STUR (11) divided those which he considered to have Marattiaceous affinities into a number of subdivisions, based on the arrangement and structure of the sporangia. His suborder Asterotheceae has the following characterization:

Sporangia ellipsoidea, apice plerumque libere subacumibata, dorso non rare in gibberum inflata, apicibusque conniventia ideoque connata, licet in sorum, synangium plus minus perfectum systementem, sessilem vel breviter pedunculatum, rotundum, stellatim congenita.

It would appear that the specimen just described belongs within STUR's suborder Asterotheceae. Within the Asterotheceae STUR placed four genera: *Asterotheca* Presl., *Scoleopteris* Zenker, *Renaullia* Stur (*Sturiella* Weiss), and *Diplozites* Goepp. (*Ptychocarpus* Weiss).

The structure of the fructification called *Ptychocarpus* (1) by WEISS (13) has been described by RENAULT (7). The fructifications are arranged in one or two series on either side of the median vein of pinnules of the *Pecopteris* type. A small number of sporangia, six to eight in the species described, *Ptychocarpus unila*, constitute a circular sorus. The sporangia are adherent to one another laterally and also to a common central receptacle, the whole sorus being imbedded in tissue which is continuous around the periphery of the sorus. Dehiscence probably occurred, according to RENAULT, through an apical pore. Very similar to this fructification is the one described by WATSON (12) under the name *Cyathotrachus altus*, which differs from *Ptychocarpus unila* in detail but not in general arrangement. These forms differ quite radically from the Illinois specimen, in the presence of the delicate tissue within which are imbedded the sporangia, in the lateral adhesion of the sporangia, and probably in the method of dehiscence.

The fructification known as *Asterotheca* (5) comprises a genus in which the sporangia, from three to eight, are grouped about a very short common receptacle, forming a circular sorus. *Asterotheca* is known to have been attached to pinnules of the *Pecopteris* type. The

sori are arranged in a single series on either side of the midvein and are sessile. The individual sporangium is ovoid in shape, with the free extremity flattened and ending in a small point. Dehiscence probably occurred by the springing apart of the sporangia when mature, the spores escaping through a vertical cleft on the inner wall of the sporangium. STUR used the generic name *Hawlia* for similar fructifications in which the sporangia were separate from one another to their bases, and places the genus in a separate suborder from the *Asterothecaceae*. KINSTON (4), however, places the Coal Measure form *Pecopteris Miltoni*, which STUR includes in *Hawlia*, in *Asterotheca*.

The general arrangement of *Asterotheca* is very similar to that of the Illinois specimen. It differs in certain important particulars. The sorus is not pediceled as is the one under consideration. The shape of the individual sporangium is usually different, that of *Asterotheca* being shorter and with the major axis usually parallel to the pinnule which supports it. It is especially different in the character of the free ends of the sporangium, which, in the form described is extended into a more or less acute apex by the elongation of the sporangial wall cells, and while there is a short apex in *Asterotheca*, does not approach the condition found in this specimen.

The genus *Scolecopteris* has the same general arrangement as *Asterotheca*. There is a relatively small number of sporangia grouped about a common receptacle, which in some species is extended to form a definite pedicel by means of which the sorus is attached to the pinnule, which is of the *Pecopteris* type. In other species the sorus is sessile. The sporangia are adherent laterally at their bases, but are free for most of their length. In the case of *Scolecopteris polymorpha*, the sporangia are laterally united for about one-third their length, which is possibly true of *S. elegans* as well. The distal ends of the sporangia are elongated into more or less acute apices, which are frequently somewhat conerescent. In some species there is a very long prolongation of the free ends, as in *S. polymorpha*.

With the species *Scolecopteris elegans*, the structure of which was described by STRASBURGER (10) in 1874, there are many points in common with the Illinois specimen. The sporangia are in groups of four and five, arranged in a single series on each side of the midvein of the pinnules of the *Pecopteris* type, the edges of which are strongly

revolute, partially inclosing the sporangia. The sporangia are distinctly pedunculate. The sporangia are conrescent toward the distal end, and, while coming to an acute point, do not have the long tapering free ends which are characteristic of *S. polymorpha*. The sporangium wall consists of more than one layer of cells, and is distinctly thicker on the outer free surface than on the radial adjacent walls. Dehiscence occurred by means of a longitudinal cleft in the sporangial wall on the inner face of the sporangium, where the wall cells are thinnest.

Scolecoperis elegans differs from the Illinois specimen chiefly in the matter of size, the sporangia of *S. elegans* measuring 0.4 mm. in diameter by 0.9 mm. in length, with spores 0.01 mm. in diameter, while the sporangia of the Illinois specimen are 0.28 mm. in diameter and about 0.7 mm. in length, with spores 0.017 mm. in diameter. Apparently the sporangia of *S. elegans* were adherent for a longer distance from their bases than in this specimen, as in no transverse section except through the very bases of the sporangia was it possible to identify a common sporangial wall such as figured by STRASBURGER for *S. elegans*.

It would seem that the differences between the Illinois specimen and *Scolecoperis*, especially *S. elegans*, are not sufficient to exclude the specimen from that genus, and it is therefore proposed to refer to the Illinois specimen as *Scolecoperis minor*, the specific name having reference to the less dimensions of the sporangia, in comparison with those species of *Scolecoperis* the structure of which has been described.

The fourth genus included in the Asterotheceae by STUR was his *Renaultia*. This form has been described originally by RENAULT (6) in 1883 under the name *Pecopteris intermedia*. Later that year STUR reviewed his work and included that fructification in his Asterotheceae under the name *Renaultia intermedia* Stur. The generic name *Renaultia*, however, had been employed slightly earlier by ZEILLER (15) for a form called *Hapalopteris* by STUR. Subsequently another generic name was proposed by WEISS (14), and the *Pecopteris intermedia* of RENAULT is known as *Sturiella intermedia* (Weiss).

The sporangia of *Sturiella intermedia* are arranged in groups of five and attached to the pinnule, which is of the *Pecopteris* type, by

a short pedicel in a single series on each side of the median vein. The sporangia were described as cylindrical, pyriform, with adjacent sides slightly flattened. The unique feature of the sporangium, however, was an apical annulus, which capped the distal end of the sporangium and extended on the free side for a distance toward the base of the sporangium. The pinnule which bore the sori had the vascular bundle in the shape of a crescent or wide U.

This description is interesting in view of the great similarity of this description with sections of *Scolecopteris minor* which have not passed exactly perpendicular through the sporangium. The longitudinal sections of *S. minor* frequently show a rather rounded apex, which, with the exception of the line for dehiscence, seems to be covered with a group of heavy walled cells which extend for a distance toward the base, dying out as it is approached. Similar sections were seen in a species of *Scolecopteris* in the British Museum. A transverse section through the prolongation of the sporangium at the distal end of *Scolecopteris minor* gives very similar views to those figured by RENAULT for *Pecopteris intermedia*, and which, in *S. minor*, consist of the heavy walled elongated cells of the sporangium wall with the cleft for dehiscence on the inner side.

Observations of RENAULT's *Pecopteris intermedia* seem to indicate that some sporangia possessed more elongated apices than those figured, and that, in one instance at least, the vascular strand of the pinnule was composed of a small circular group of very small tracheids in place of the characteristic horseshoe-shaped bundle figured. While the hairs attached to the sporangia of RENAULT's specimen are unique and not duplicated in known species of *Scolecopteris* with structure preserved, it would appear that there is perhaps less distinction between these genera than usually considered.

The natural affinities of *Scolecopteris* are not positively known, but although the relation of all fernlike fructifications has seriously been questioned as the result of the discovery that some at least were microsporangia of early seed plants, opinion regarding *Scolecopteris* in general is the same as that given by STRASBURGER in 1874 in regard to *Scolecopteris elegans*:

ZENKER'S (16) *Scolecopteris elegans*, according to the formation of its sorus, certainly belongs to the Marattiaceae, and in fact, comes nearest the genus

Marattia in the form of the sporangia of which the sori are composed, while it approaches the genus *Kaulfussia* in the circular grouping of the sporangia, and finally resembles the genus *Angiopteris* in the fact that the sporangia become free in their upper part. In the mode of dehiscence of the individual sporangia, *Scolecopteris* agrees with all three genera mentioned, but the similarity with *Marattia* is again the most striking, to which it also bears the greatest resemblance in the structure of the sori.

To which SCOTT (8) adds, "So far as the pedicellate sori are concerned [and *S. minor* belongs here] the agreement is especially close with the sub-genus *Eupodium* of *Marattia*."

STRASBURGER, of course, wrote before the discovery of that extinct group of early seed plants, the Cycadofilicales, which has necessitated so much revision of thought in regard to fernlike impressions and fructifications. Whereas formerly it was naturally considered that a plant with fernlike foliage or sporangia belonged to the true ferns, it is now known that some species of practically every form genus of fernlike foliage belonged to seed bearing plants, and that the supposed fern sporangia were the microsporangia of seed plants. *Pecopteris* is no exception. Seeds have been found attached to at least one species, *P. Pluckenti* (3). Also *Crossotheca*, the bilocular sporangium of *Lyginopteris*, is listed by KIDSTON (4) as having been attached to three species of *Pecopteris*. It is true that no unilocular sporangium, such as *Scolecopteris*, is definitely known to have been the microsporangium of a seed plant, but according to SCOTT (9), "It is however probable that synangia such as those of *Telangium Scotti*, in which the sporangia are unilocular, may also have belonged to Lyginopterideae, and in that case an analogy with *Scolecopteris* would be evident; at present, however, our knowledge is too imperfect to justify further speculation."

More recently, however, unpublished researches by HALLE, according to a letter from Dr. SCOTT, gives excellent evidence that a new Pteridosperm bore seeds attached to the frond of a *Pecopteris* type of foliage similar to that on which has been found the *Asterotheca* type of sporangia.

On the other hand, the evidence that *Scolecopteris* is the fructification of a true fern has been strengthened by the anatomical work on *Psaronius*, which is generally considered to be the stem of an

arborescent fern with Marattiaceous affinities. GRAND'EURY (2) was convinced that the fronds of *Psaronius* were of the *Pecopteris* type, such as those which bear *Asterotheca* and *Scolecopteris*. This would indicate that these fructifications, in some instances at least, belonged to the true ferns. It is interesting to note in connection with the question of the relationship of *Pecopteris* fronds and fructifications with the stem of *Psaronius*, that closely associated with *Scolecopteris minor* in the petrifications were numerous *Psaronius* rootlets, although it is well understood that close association in coal ball material is absolutely no evidence for previous organic connection.

In view of the close relationship between the *Asterotheca* and *Scolecopteris* type of fructification, however, conclusive evidence that the plant on which the *Asterotheca* type of sporangia occurs also bore seeds, as indicated by HALLE's investigations, would seem to warrant the assumption that *Scolecopteris* likewise is the male fructification of a member of the Cycadofilicales, conclusive evidence of which, however, must await further investigation.

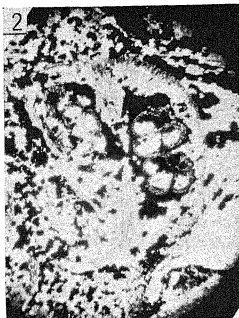
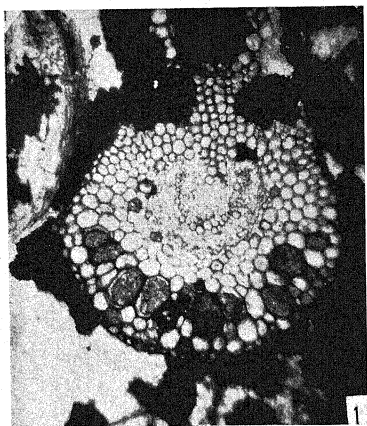
Acknowledgment is due to Dr. A. C. NOÉ, whose generosity in regard to petrifications and laboratory facilities made this paper possible, and to Dr. FREDDA REED for certain critical comments during the preparation of the manuscript.

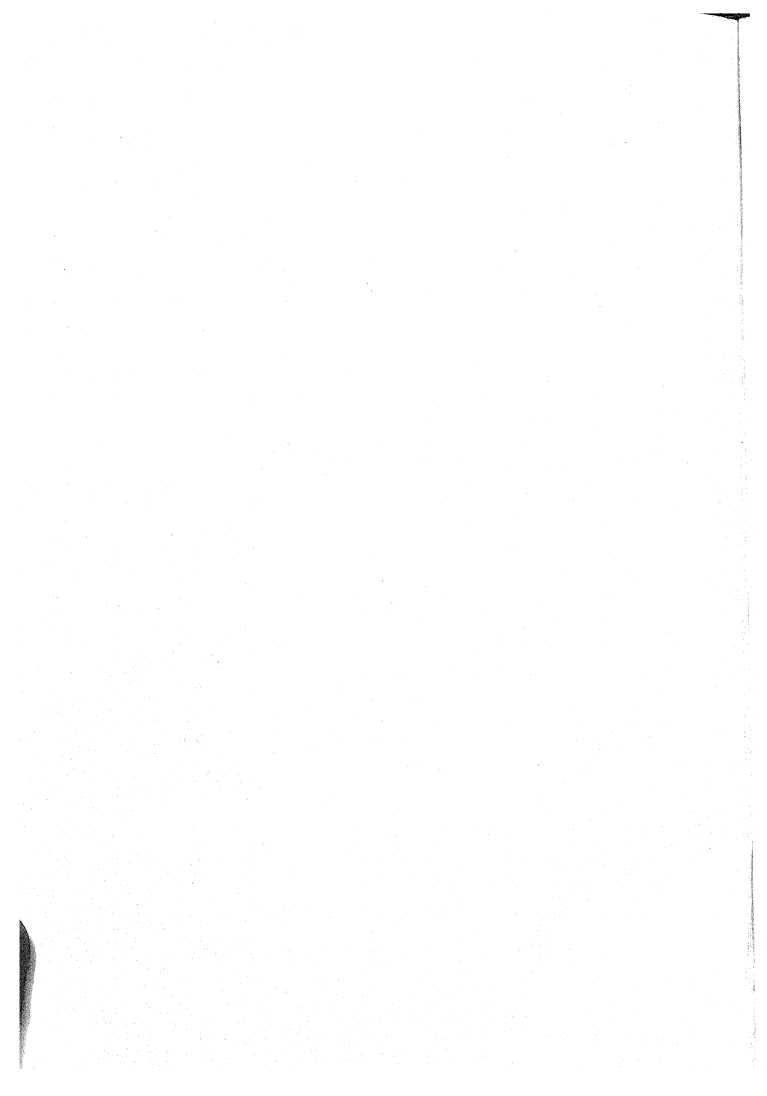
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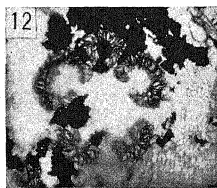
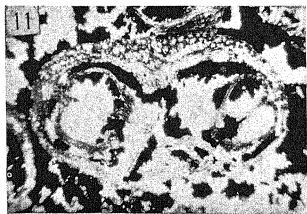
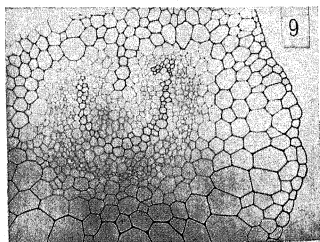
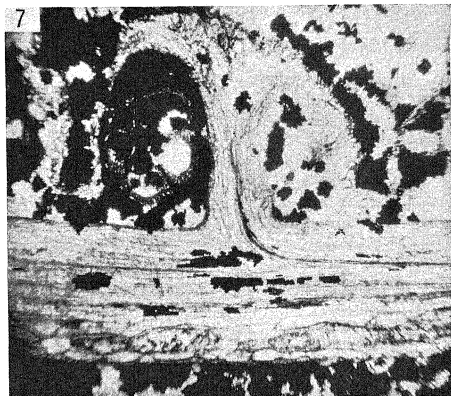
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EXPLANATION OF PLATES XXIII, XXIV

PLATE XXIII

FIG. 1.—Transverse section of ultimate rachis, with characteristic horse-shoe-shaped vascular bundle; $\times 35$.

FIG. 2.—Section parallel to surface of pinnule: to right of midvein are two sori in transverse section, one with four sporangia and one with five; $\times 16$.

FIG. 3.—Transverse section of portion of pinnule showing furled edge of pinnule and attachment of sorus; $\times 20$.

FIG. 4.—Oblique section through ultimate rachis with pinnule cut parallel to surface, showing venation and one sorus in transverse section; $\times 16$.

FIG. 5.—Longitudinal section of sorus showing parts of three sporangia and method of attachment of sorus; $\times 20$.

FIG. 6.—Transverse section of ultimate rachis through two pinnules, parts of four sori shown; $\times 35$.

PLATE XXIV

FIG. 7.—Longitudinal section of ultimate rachis with one pinnule attached, showing single pinnule trace clearly; to left of midvein of pinnule is sorus in transverse section, in lower sporangium of which are spores; $\times 35$.

FIG. 8.—Transverse section of sorus of five sporangia; $\times 27$.

FIG. 9.—Camera lucida drawing of fig. 1.

FIG. 10.—Section of pinnule in plane of surface, showing type of venation.

FIG. 11.—Transverse section of pinnule: edges of pinnule curved, partially inclosing the two sori; common attachment of each sorus evident; $\times 27$.

FIG. 12.—Transverse section of sorus near distal ends of sporangia; dehiscence occurred through longitudinal slit on inner surface of each sporangium; $\times 25$.

LEGUME INOCULATION AS INFLUENCED BY STOCK AND SCION

THOMAS E. RICHMOND

(WITH TWO FIGURES)

Introduction

That the nodule bacteria of *Phaseolus lunatus* are distinct from those of *P. vulgaris* has been shown by WHITING and HANSEN.¹ This probably is the first case on record in which all the species within a given plant genus are not inoculated by the same nodule organism. As yet no fundamental relationship has been reached upon which a satisfactory grouping of the nodule organism for inoculation purposes can be made.

As it is possible to grow to maturity a lima bean top on a navy bean root, or a navy top on a lima root, an entirely untouched method of investigating the relation of the host plant to its nodule-producing organism is opened for study. The answer to such questions as follows might throw light upon the relationship of the host plant to its nodule organism: (1) Which organism, that specific to the lima or to the navy bean plant, will produce nodules upon a navy root when grafted with a lima top? (2) Will the nitrogen content of a lima top upon an inoculated navy root be the same as that of a normally inoculated lima plant? (3) Will the processes of photosynthesis and metabolism be the same in a grafted plant as in the normal plant? (4) Will the nitrogen compounds found in the seeds from uninoculated, normally inoculated, and grafted plants be the same? (5) Will the seeds produced by grafted plants produce plants that still retain the power of selection between the nodule organisms?

Preliminary work

In most of the following work sterilized seeds were planted in quartz sand and allowed to grow to a height of 6-8 inches before washing out and making the desired grafts. Grafting was performed

¹ WHITING, A. L., and HANSEN, R., Cross inoculation studies with the nodule bacteria of lima beans, navy beans, cowpeas, and others of the cowpea group. *Soil Science* 10: no. 1. 1920.

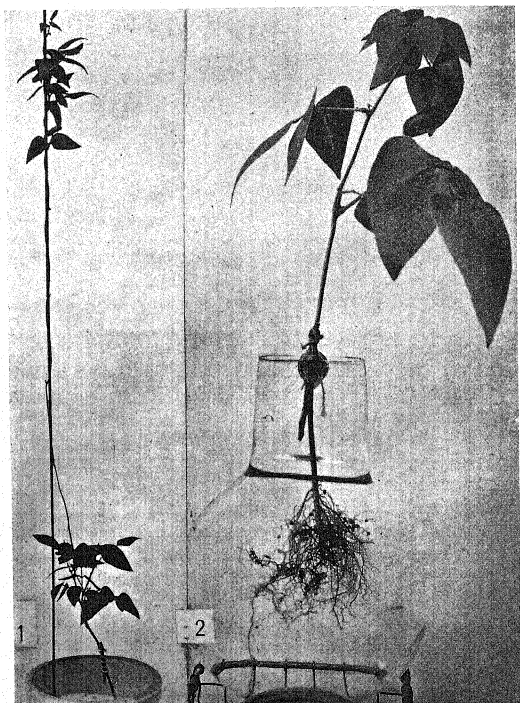
by cutting half the stem away for a distance of about 4 inches. Lima and navy seedlings thus treated were placed so that the two cut surfaces were together and bound firmly to each other with string. After the seedlings were securely fastened, the united stem was punctured in several places with a needle. The combined plants were then replanted in quartz sand in a gallon jar and tied to a support. In about a week's time the stem of one of the two beans was nearly cut in two between the root and bottom of the graft, and at the same time the top of the other plant was cut off. In a few days' time the cut root was severed and removed from the jar. This would then leave a lima bean root with a navy bean top, or a navy root with a lima bean top, as the graft happened to be made. In all cases the plants were watered with tap water, which was found to be not only free of the nodule organism, but also contained sufficient plant food elements, with the exception of nitrogen, to grow plants to maturity. After a successful graft had been made, the nodule organism to be used was added to the jar and the plant allowed to grow for the required time.

EXPERIMENT 1.—The first graft made was a lima top upon a navy root. This was successful and the nodule organisms of the lima bean added to the jar June 1, 1924. Planted in this same jar were sterilized seeds of both lima and navy beans, which were allowed to grow as normal plants in order to test the purity of the nodule organism used. The grafted plant grew well, and upon examination of the check plants in July it was found that the navy bean was uninoculated while the lima bean was well inoculated. This indicated that the lima bean culture used caused nodulation upon lima, but not upon navy bean, and was therefore a pure culture of the lima bean organism.

The grafted plant grew to a height of several feet, and produced well developed pods with several beans in each (fig. 1). This plant was washed out September 2, 1924, and it was found that the root, while free from nodules, had been stimulated to a marked degree. The dry weight and total nitrogen determination for this plant were as follows:

Oven-dry weight of top (except seeds)	2.40 gm.
Oven-dry weight of roots	1.62 gm.
Weight of nitrogen in top	.49 mg.
Weight of nitrogen in roots	.34 mg.

As this plant had matured several beans, and still had a nitrogen content of 83 mg., the fact is evident that it must have been able to obtain nitrogen even though no nodules were formed upon its roots.



FIGS. 1, 2.—Fig. 1, lima bean top-navy bean root, treated with lima bean nodule organism; leaves removed from part of stem; fig. 2, lima bean top growing upon an inoculated navy bean root.

The nitrogen content of the original bean, as determined by the analysis of similar beans, was about 12-15 mg. The normal navy bean plant grown in the same jar, as a check upon the organisms used, was dying of nitrogen starvation when removed for examination of its roots for nodules, showing that the nitrogen added in the bacterial culture and tap water was not the source of nitrogen available to the grafted plant.

EXPERIMENT 2.—In this trial, grafted plants were inoculated with the organism common to the root of the graft, and it was found in every case in which the navy bean organism was added to a jar in which a lima top was growing upon a navy root, that inoculation occurred and the grafted plant grew to maturity and produced seed. The reciprocal grafts and inoculations with lima bean cultures also produced nodules in all cases.

EXPERIMENT 3.—In this case a lima bean top was grafted upon a navy bean root that already had nodules upon its roots. This graft was successful and the plant grew well. The graft was made June 1, and washed out August 8, 1924 (fig. 2). The plant had several mature beans and was normal in all respects. The root was in good shape and the nodules active. This lima bean top had been able to obtain nitrogen through the inoculated navy bean root.

EXPERIMENT 4.—The question of the behavior of beans grown upon grafted plants toward the nodule organism was next investigated. Navy beans which had grown upon lima roots, and lima beans which had been grown upon navy roots were used in this experiment. The sterilized beans were planted in quartz sand and inoculated June 9, 1924, as indicated:

	Nodulation
Modified navy bean seed plus navy bean bacteria	+
Modified navy bean seed plus lima bean bacteria	+
Modified lima bean seed plus navy bean bacteria	+
Modified lima bean seed plus lima bean bacteria	+
Normal lima bean seed plus navy bean bacteria	—
Normal navy bean seed plus lima bean bacteria	—

These plants all grew well and were washed out July 18. Nodules were found upon the roots of all the plants except the checks, but it was at once apparent that the nodules upon the navy bean roots inoculated with lima bean organisms and those upon the lima beans

inoculated with navy bean organisms were quite different from normal nodules. They occurred only upon the main root, which appeared to be enlarged and cracked at the point of union, and were whitish in color and flat. The plants, however, gave no appearance of nitrogen starvation.

As normal lima and navy beans inoculated and grown in this same way gave no signs of nodule formation, it appears that the beans grown upon grafted plants have in some way been modified so that they are no longer able to differentiate between the nodule organisms of the lima and navy bean.

Summary

1. When a lima bean top is growing upon a navy bean root, in quartz sand, a pure culture of lima bean bacteria will not cause the formation of nodules upon the roots of the grafted plant, but the roots are stimulated, the plant grows to maturity, and appears to be able to obtain atmospheric nitrogen.

2. When such a graft is inoculated with a navy bean culture, nodules are formed and the plant grows to maturity. The navy bean organisms in the roots apparently are able to furnish nitrogenous compounds to the lima bean tops, in exchange for carbohydrates synthesized by a top not normal to the nodule organism used.

3. Similar results are obtained with the reciprocal grafts and inoculations.

4. A lima bean top grafted upon an inoculated navy bean root grew to maturity and developed seeds.

5. When seeds are produced by a grafted plant, either lima or navy, they are so modified that plants grown from such seeds no longer have the power of selective adaptation for the specific nodule organism common to it, but are inoculated by either the lima or navy bean organism.

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CURRENT LITERATURE

BOOK REVIEWS

Manual of plant diseases

A wealth of well selected and judiciously arranged material is packed into HEALD's new volume on plant diseases.^{*} It will be useful to the student primarily as a reference book, since treatment of the material in the main is encyclopedic. As a starting point in dealing with various non-parasitic diseases, the author briefly discusses the requirements and reactions of the normal or healthy plant. This introduction of some of the fundamental principles of the subject greatly enhances the value of the work as a text. A considerable part of the value of the book for reference lies in the accurate and up-to-date presentation of the material, the painstaking reference to sources for statements, and the excellent bibliographies which close each chapter. Illustrations and diagrams are well selected and well done, and increase the utility of the volume.

In defining the field of plant pathology the author refers to phytopathologists as "the trained plant doctors, the 'medicine men of agriculture,' whose final goal is successfully to prevent or control plant diseases." This statement sounds the keynote of the book. The material is presented from the point of view of training the "farmer, gardener, or professional plant doctor" to make diagnoses and to employ or recommend proper control measures. It would have been well to point out that the field of phytopathology also includes investigations in the fundamental biological aspects of the subject, such as the phenomena of pathological anatomy, parasitism, and immunity, for the history of phytopathology parallels that of medicine, in that, in the main, like medicine it is founded not on empiricism but on scientific investigation, much of which has its origin in pure intellectual curiosity. Even if we ignore this failure to point out the real field of the science of phytopathology as contrasted with its art, the comparison is unfortunate. Whatever success the plant pathologist has had in controlling plant diseases has been by prevention rather than by cure or medication. The method and goal of the phytopathologist are more akin to those of the worker in public health than to those of the medicine man.

By including the pathological conditions of plants due to non-living factors (non-parasitic diseases), the author departs from the usage of the older American writers on plant pathology. This is a decided gain. The book does not go all the way, however, in defining the field of phytopathology. The only reference to the pathological conditions of plants induced by animals, save that due to nematodes, is an attempt to justify the present usage of assigning

^{*} HEALD, F. DEF., *Manual of plant diseases*. Svo. xiii+891. figs. 272. New York: McGraw Hill Book Co. 1926. \$7.00.

the field to the economic zoologist and entomologist who are only secondarily interested in the affected plant. Nine chapters (176 pages) are devoted to non-parasitic diseases; one chapter (55 pages) to virus diseases; thirteen chapters (502 pages) to diseases due to the activity of bacteria and fungi; one chapter (21 pages) to pathological conditions induced by parasitic seed plants; one chapter (22 pages) to diseases induced by nematodes; and one chapter (20 pages) to the structure of fungi with a slight reference to their physiology. The use of such phrases as "Pondscum parasites" instead of Chytridiales, or "Black molds and allies" instead of Zygomycetes in chapter captions has nothing to commend it, since the manual was not designed for popular appeal.

The book is well printed and illustrated, and should go a long way toward meeting the demand for a modern text of plant pathology.—G. K. K. LINK.

Gymnosperms

The second edition of the gymnosperm section of ENGLER and PRANTL'S *Die Natürlichen Pflanzenfamilien*, just issued by ENGLER,² is much enlarged and improved, with the 127 pages and 82 figures of the first edition increased to 441 pages and 240 figures. In the first edition only four families, the Cycadaceae, Cordaitaceae, Coniferae, and Gnetaceae were recognized; while in the second edition the material is treated under seven orders: Cycadofilices, Cycadales, Bennettitales, Ginkgoales, Cordaitales, Coniferae, and Gnetales. PILGER supervised the whole work and wrote the living Cycadales, Ginkgoales, and Coniferae, while KRAÜSEL added the fossil members of these orders. GOTHAN wrote the Cycadofilices, and MARKGRAF the Gnetales.

The family ending "aceae" is used in the Coniferae, so that we have Cupressaceae instead of Cupressineae, etc., and a similar raising of rank appears in other places. It is to be regretted that in a work which is so widely used, there is so little consistency in the endings. The ending "ales" has become almost as well recognized as an ordinal ending as "aceae" is for a family ending. Cycadofilicales and Coniferales would have made the series consistent.

In the Cycadales the number of genera remains 9 and the number of species about 83; but the number of pages dealing with this group has been doubled. The treatment, even from the morphological standpoint, reflects much of the progress of the past 30 years. The keys, which in the old edition were based upon cone characters, are here based primarily upon vegetative characters, which make them more serviceable, because cycads usually are not in fruit. Vegetative characters could have been used to a still greater extent.

The greatest changes and largest additions have been made in the Coniferales, which now have 286 pages instead of 88, and 107 illustrations instead of 58. Seven families are recognized, the Taxaceae, Podocarpaceae, Araucariaceae, Cephalotaxaceae, Pinaceae (including Abietoideae and Pinoideae),

² ENGLER, A., *Die Natürlichen Pflanzenfamilien. Gymnospermae*. pp. 447. figs. 240. Leipzig: Wilhelm Engelmann. 1926.

Taxodiaceae, and Cupressaceae; a very different sequence from that of the old edition, which began with the Pinoideae and ended with the Taxaeae. The Cupressaceae are probably a very advanced group, but many will not approve the position assigned to the Taxaceae or the placing of the Araucariaceae between two taxad groups.

While primarily the volume is a taxonomic work, a large proportion of the space is given to morphology, phylogeny, and geographic distribution, and the lists of literature contain many references to these phases of the subject.

One of the most important improvements is the rather full account of extinct forms, which received a very inadequate treatment in the old edition. Many of the illustrations are new, and some of those in the Cycadales are taken from SCHUSTER's forthcoming monograph of this order.—C. J. CHAMBERLAIN.

Ion effects in colloidal systems

All biologists will welcome the publication of the little volume by MICHAELIS³ embodying lectures delivered in 1924 at various universities in the United States. Most of the book is taken up with a discussion of adsorption and electrical double layers, with emphasis on the importance of a correct understanding of these, if the biologist is to understand the life processes of the cell. The author considers of course that the condition of the colloidal material of the cell controls its vital functions, for example, respiration, irritability, etc. The condition of the colloidal material depends upon its electrical charges, however, hence the importance of a correct understanding of electrical double layers and adsorption.

The volume opens with a statement of the author's intention in regard to the use of the term adsorption. The term is used to include not only cases of real chemical union, that is, where the compound adsorbed is molecularly combined with the adsorbing medium, but also the cases where the union is more physical, the compound adsorbed accumulating in the more superficial layers of the water. Chapter II takes up certain electrical phenomena resulting from the adsorption of ions. Electromotive forces, cataphoresis, and endosmosis are discussed. Next is considered the origin of electrical double layers. Three possibilities are discussed, double layers resulting from appositional adsorption, those formed as a result of the dissociation of colloidal particles, and those formed at the surface of chemically inert materials such as collodion, cellulose, etc. There follows a discussion of the adsorption of ions by charcoal, and a consideration of the quite peculiar place held by charcoal as an adsorbing medium, this being mainly due to the fact that, in contrast with most substances, it has a great power of adsorbing non-ionized, capillary active compounds. The analogy of this peculiar property to conditions in the living cell is discussed. Next is considered the Donnan equilibrium. The author reconciles the production of an electrical double layer in this phenomenon with the other types of

³ MICHAELIS, L., *The effects of ions in colloidal systems*. pp. 108. Baltimore: Williams and Wilkins Co. 1925.

electrical double layers by regarding it as belonging to the case of the production of an electrical double layer by the dissociation of colloidal particles. The last two chapters consider the lyotropic effects of ions, and the effects of mixtures of ions.

The discussion is compact, and one needs to have done considerable reading on the points taken up fully to understand them. The author fully appreciates the insufficient state of our knowledge of adsorption and electrical double layers. There is a proper balance between the statement of facts as revealed by experiments and the theoretical part, which looks ahead and calls for further experimentation. The book should be very helpful to biologists, and should stimulate further research in this important and difficult field.—S. V. EATON.

Plant nutrition and crop production

The Hitchcock Lectures at the University of California in 1924 were given by Sir JOHN RUSSELL, the distinguished director of the Rothamsted Station. These lectures have been published in book form,⁴ and are worth possessing by anyone interested in any way in the scientific side of plant nutrition and the production of crop plants. There are five lectures, the first of which deals mainly with the background of history of the study of plant nutrients. Naturally this follows along the same lines as the historical account in his previous book on *Soil conditions and plant growth*.

The second chapter is entitled "Positive science and exact demonstration." It emphasizes the value of quantitative studies of plant behavior to soil factors and other influential environmental conditions. The reference to auximones does not fit in so well with the title of the chapter, as it is not yet demonstrated that there is such a thing as an auximone. BOTTOMLEY's results have failed of confirmation in a number of studies, and it is certain that his original cultures were poorly balanced as to minerals. Likewise, the auximones without doubt contained balancing minerals. The third lecture discusses decay and the living plant, and considers the function of organic matter in the soil and some of the organisms occurring there, and particularly the relations of bacteria and Dimastigamoebae. The fourth discourse concerns the question as to whether the microorganic population can be controlled or utilized. Inoculation, sterilization, and control through favoring the helpful and discouraging the harmful forms of living things are excellently discussed. The final chapter deals with the great complexity of the relations of plants as organisms with the soil, itself enormously complex.

The book is written in a charming style, and the 21 plates and 37 figures give it an unusually fine appearance. The plate showing pictures of GILBERT, LAWES, LIEBIG, and BOUSSINGAULT will be appreciated by all possessors of the volume. It is in every way an excellent work which should have a wide distribution.—C. A. SHULL.

⁴ RUSSELL, SIR JOHN, *Plant nutrition and crop production*. 8vo. pp. x+115. University of California Press. 1926. \$2.50.

The Aspergilli

A much needed book on the the genus *Aspergillus* has been written by THOM and Miss CHURCH.⁵ The book has grown out of the extensive laboratory work with members of the genus *Aspergillus* begun by THOM in 1904, when he undertook investigation of the molds which play a rôle in food handling. In the course of their studies, the authors examined the original description of all save three or four of the sixty odd species of *Aspergillus* listed in the book. The book will be extremely useful to workers in many fields. Some Aspergilli are favorites for biochemical studies; some play an important rôle in industrial fermentations; some cause disease of animals; some play an important rôle in the spoilage of fruits, vegetables, and nuts in transit and in storage; and finally, many of them play a provokingly persistent and important rôle as "weeds of the culture room." A history of *Aspergillus* is followed by discussion of its morphology, physiology, biochemistry, and taxonomy. There are three useful keys: one based on color of the heads and stalks, the second a synoptical key based upon actual cultures of Aspergilli, and the third an abbreviated form of the second. Separate chapters are devoted to such topics as Culture of Aspergilli; Physiological and Biochemical studies; Enzymic and fermentative activity of Aspergilli and their Industrial significance; and Pathogenic aspects.—G. K. K. LINK.

British lichens

All students of the lichens in America or elsewhere make constant use of SMITH's admirable works on the lichens of Great Britain. The first part of the first edition was the work of Rev. JAMES CROMBIE, but after his death in 1906 the task was taken over by ANNIE LORRAIN SMITH, who issued the second part of the first edition in 1911. The first part of the second edition appeared in 1918, and the concluding (second) part of this edition has now appeared.⁶ The author in 1921 also published her extremely useful *Handbook of British lichens*, which has had very large use by students of lichens here and abroad.

The new edition shows no fundamental changes, although there are some alterations in the grouping of species, especially in *Lecidea*. The present volume contains descriptions and habitat data concerning some of the Cyclocarpineae not considered in the first part, especially the Lecideaceae. There are also treated here the Graphidineae and the Pyrenocarpeae. In the latter pages is a list of microfungi recorded by British authors as lichens, also an appendix to Part I with emendations and additions. The work concludes with a most admirable glossary of terms used in lichenology, and an index which includes not only generic but also specific names and synonyms.—H. C. COWLES.

⁵ THOM, C., and CHURCH, MARGARET B., *The Aspergilli*. 8vo. pp. ix+272. figs. 13. pls. IV. Baltimore: Williams & Wilkins Co. 1926. \$5.00.

⁶ SMITH, ANNIE LORRAIN, *A monograph of the British lichens*. A descriptive catalogue of the species in the Department of Botany, British Museum. Part II. 2d ed. revised. pp. ix+447. pls. 63. London: British Museum (Nat. Hist.). 1926.

A naturalist in East Africa

An interesting account of his travels in Uganda has been published in book form by CARPENTER.⁷ The author, who was medical officer at various military posts, spent his leisure in studying the natural history of the region. Particular attention was given to the insect life, problems of protective coloration being among the topics discussed, and brief notes on the vegetation are also recorded. The excellent illustrations range from photographs of butterflies, giant lobelias, and bamboo forests, to views of Victoria Falls.—G. D. FULLER.

NOTES FOR STUDENTS

Functions of transpiration.—Various functions for transpiration have been given from time to time. By some it has been considered a process essentially useless or even injurious to the plant, because of the large amount of water lost in the process. At the other extreme are those who consider transpiration a process very beneficial; in fact, a process without which the plant could not live. Some of those who hold this view speak of two main benefits accruing to the plant from transpiration, the cooling effect of the process, and the importance of the process in the transfer of salts into the plant and from the roots to the growing parts.

CURTIS⁸ has given an excellent summary of the significance of transpiration. As to the cooling effect of transpiration, reference is made to several papers, among them a paper by CLUM,⁹ showing that the temperature of the leaf is reduced by transpiration not more than from 1° to 5° C. CLUM vaselined certain leaves and compared the temperature of these with unvaselined leaves. The leaves in which transpiration was checked by vaseline were only 2°–4° C. warmer than the unvaselined leaves. In another paper, CLUM¹⁰ shows that the temperature of a leaf may be reduced as much as 7° C. by shading it, thus illustrating the efficiency of convection and radiation in cooling the leaf. CURTIS and CLUM regard light intensity, angle of incidence, convection and radiation, and air currents as having a much greater effect on the temperature of the leaf than the cooling effect of transpiration. It may well be that the loss of heat by conduction and convection is rapid enough to prevent undue heating when transpiration is checked; but, in view of the large amount of water transpired by plants and the high heat of vaporization of water, it would seem that under

⁷ CARPENTER, G. D. H., *A naturalist in East Africa*. 8vo. pp. 187. pls. 8. figs. 23. 3 maps. Oxford Univ. Press; American branch, New York. 1925. \$5.

⁸ CURTIS, O. F., What is the significance of transpiration? *Science* 63:267–271. 1926.

⁹ CLUM, H. H., The effect of transpiration and environmental factors on leaf temperatures. I. Transpiration. *Amer. Jour. Bot.* 13:194–216. 1926.

¹⁰ ———, II. Light intensity and the relation of transpiration to the thermal death point. *ibid.* 217–230. 1926.

normal conditions, when transpiration is not checked, it accounts for the dissipation of much of the solar energy absorbed by plants. BROWN and ESCOMBE's balance sheet of the solar energy falling on the leaf certainly gives an important place to transpiration in the dissipation of this energy. According to their viewpoint, transpiration would not be a necessary process for keeping down the temperature of the leaf, for when transpiration is checked, conduction and convection cool the leaf, but this does not alter the fact that under normal conditions transpiration may account for the use made by the leaf of much of the energy absorbed by it.

In regard to the importance of transpiration in relation to the movement of salts into the root, and from the root to the growing parts of the plant, reference is made to work of MUENSCHER showing that high transpiration does not cause increased movement into the plant and from the roots to the tops. While salts certainly enter the root from the soil solution and pass through the cortical cells of the root to the tracheae independently of the water, yet, as CURTIS points out, if the salts pass by mass movement in the transpiration stream from the roots to the leaves, it is hard to see why this does not cause increased absorption by the root and therefore a concentration of salts in the tops. On the other hand, if the salts are not carried along by the transpiration stream from the roots to the leaves, it is hard to see how the growing parts of the plant are provided with enough salt material.

Other points included by CURTIS in his summary are effects in the plant of a reduction of the water content of the plant, effects of a reduction of the water content of the soil, and certain effects that are often falsely ascribed to transpiration.

As CURTIS points out, most of the work on transpiration has had to do with the amount of water transpired, and the factors affecting this water loss. Much more experimental work is needed on the functions of transpiration. CURTIS has done a big service in again opening up this subject.—S. V. EATON.

Taxonomic notes.—In his fourth paper on "new and noteworthy fungi," DEARNESS¹¹ has published 9 new species of Discomycetes in 5 genera, 18 new species of Pyrenomycetes in 15 genera, one of which (*Phragmodothidea*) is new, and 12 new species of Deuteromycetes in 9 genera.

MISS WEEDON,¹² in publishing the results of her collection of fungi near St. Petersburg, Florida, in addition to 4 new species, has described 2 new genera, *Exophoma* on *Magnolia*, and *Macrophomopsis* on *Dracaena*.

In continuation of his revision of the grasses of Japan, HONDA¹³ has described 7 new species, 4 of them belonging to *Calamagrostis*.

¹¹ DEARNESS, JOHN, New and noteworthy fungi. IV Mycologia 18:236-255. 1926.

¹² WEEDON, AMY G., Some Florida fungi. Mycologia 18:218-223. 1926.

¹³ HONDA, M., Revisio Graminum Japoniae. X. Bot. Mag. Tokyo 40:317-327. 1926.

KOIDZUMI¹⁴ is publishing a series of contributions to the flora of eastern Asia, the knowledge of which is increasing rapidly. In the present paper, among the numerous additions to this flora, there are described new species of *Fraxinus*, *Salix*, and *Hydrangea*.

TIFFANY¹⁵ has published the results of collections of filamentous algae made in northwestern Iowa during four seasons. It is a region of lakes, swamps, and streams that are rich in freshwater algae. The report includes 35 Myxophyceae and 165 Chlorophyceae, by far the largest genus being *Oedogonium*, with 68 species, one of which is described as new. The majority of the forms have not been reported for Iowa, and 6 forms of *Spirogyra*, 15 of *Oedogonium*, and 3 of *Bulbochaete* have not been recorded previously from North America.

MERRILL¹⁶ has published a third paper recording additions to the Philippine flora. The species considered are mainly from the Sulu Archipelago, from two islands that had never been explored botanically. The paper lists 47 species as new to the Philippine flora, 32 of which, distributed among 30 genera, are described as new. Eight of the genera are recorded for the first time from the Archipelago.

JACKSON¹⁷ has begun the publication of the collections of rusts made in South America by E. W. D. HOLWAY during two excursions, which included Ecuador, Bolivia, Peru, Chile, Brazil, and Argentina. The rusts are presented by families of the host plants, representing 49 species on 71 hosts, 13 of the species being described as new.

PERCIVAL¹⁸ has described 21 new varieties of emmer wheat obtained from Abyssinia, Somaliland, Morocco, and Transcaucasia, grown in the highlands of the country at an elevation of about 10,000 feet. In the same paper there are described also 7 new varieties from regions of Asia Minor.

HANSFORD¹⁹ has published a very full account of the genus *Fusarium* as found in Jamaica. The work was done in connection with experimental work on the Panama disease of the banana, which necessitated an examination of the *Fusarium* flora of a large number of soil samples. As a result, 35 species and varieties are recognized and classified.

¹⁴ KOIDZUMI, G., Contributions ad cognitionem Florae Asiae Orientalis. Bot. Mag. Tokyo 40:330-348. 1926.

¹⁵ TIFFANY, L. H., The filamentous algae of northwestern Iowa, with special reference to the Oedogoniaceae. Trans. Amer. Micr. Soc. 45:69-132. 1926.

¹⁶ MERRILL, E. D., Additions to our knowledge of the Philippine Flora. III. Phil. Jour. Sci. 30:389-430. 1926.

¹⁷ JACKSON, H. S., The rusts of South America based on the Holway collections. I. Mycologia 18:139-162. 1926.

¹⁸ PERCIVAL, J., Some new varieties of wheat. Jour. Bot. 64:203-210. 1926.

¹⁹ HANSFORD, C. G., The Fusaria of Jamaica. Kew Bull. Miscell. Inf. no. 7. 1926.

PIPER²⁰ has published the results of his studies of certain groups of Phaseolineae (Fabaceae). Twelve genera are presented, two of which are new (*Condylostylis* and *Alepidocalyx*). Much the largest genus is *Phaseolus*, with 95 species, 36 of which are described as new.

SMALL²¹ has described a new species of *Zamia* (*Z. silvicola*) from "peninsular Florida," said to be the most robust *Zamia* in Florida.

CURTIS²² has described a new genus (*Claustula*) of Phalloid Fungi, which he collected in New Zealand. The systematic relationship is not clear, since sufficiently young material to trace the early development was not available. The author's conclusion is that "the fungus is either an unusual member of the Phallineae or has affinities with that group."—J. M. C.

Artificial selectively semipermeable membranes.—Selective semipermeability is well known among plant membranes, both living and lifeless, but the causes of selective behavior have never been fully explained. The production of artificial membranes which duplicate the type of behavior of seed coats and plasmatic membranes is a rather remarkable achievement. KAHLENBERG has been interested in permeability and osmosis for many years, and has now²³ been able by dialysis to separate crystalloids from one another. He even claims that it is possible to separate colloids from crystalloids by having the colloids pass through the dialyzing membranes, leaving the crystalloids behind, by proper choice of septa and solvents.

For nonaqueous solutions the septum used is rubber damask and the solvent pyridine, which is unique in being miscible in all proportions in water, and at the same time equally miscible with other organic solvents which do not mix with water. Sulphur, naphthalene, and camphor, for instance, all dissolve in pyridine, and also in hydrocarbons. Since rubber behaves like a hydrocarbon, these substances in solution in pyridine will pass the rubber septum into pure pyridine on the opposite side of the membrane. Silver nitrate, lithium chloride, and sucrose, although soluble in pyridine, will not dissolve in hydrocarbons, and will not penetrate rubber. It follows, then, that a solution of sulphur and lithium chloride in pyridine can be separated by dialysis through the rubber membrane, sulphur passing and leaving the lithium salt behind. All of the substances insoluble in rubber can be removed quantitatively from those not soluble in rubber in this way, in from two days to three weeks.

²⁰ PIPER, C. V., Studies in American Phaesolineae. Contrib. U.S. Nat. Herb. 22: 663-701. 1926.

²¹ SMALL, J. K., Cycads. Jour. N.Y. Bot. Gard. 27:121-129. 1926.

²² CURTIS, K. M., The morphology of *Claustula Fischeri*, gen. et sp. nov. A new genus of Phalloid affinity. Ann. Botany 40:471-477. 1926.

²³ KAHLENBERG, L., On the separation of crystalloids from one another by dialysis. Philosophical Mag. (7th series) 1:385-394. 1926.

For separation of solutes in aqueous solution a membrane of lanoline supported by parchment paper or on a fine quality of China silk was used. They were made by soaking the silk in a chloroform solution of lanoline. Collodion membranes were impracticable as a basis for lanoline films, although they can be made. Pieces of lanoline silk tied over thistle tubes in the usual way were used to separate crystalloids by dialysis. Cane sugar, lactose, and dextrose were found not to pass even in two weeks' time, while urea, boric acid, and sodium chloride went through more readily. The speed of passage of urea and sodium chloride differed so much, however, that these were separated fairly well by dialysis. Hydrochloric acid required seven days to pass perceptibly. Through these membranes the author reports separation of urea from mannose, the latter substance not passing through the membrane until after 31 days. Sodium chloride was separated from nickel chloride, boric acid from cane sugar, and urea from sodium chloride.

The ingredient of lanoline responsible for this behavior is found to be cholesterol, and the plant phytosterols were found to possess the same properties. The idea proposed is that "it is extremely delicate films of these sterenes that give living cells their wonderfully selective osmotic properties." No other waxy constituents were found capable of giving these properties to films of silk or parchment paper. The hypothesis is worthy of consideration in connection with plasmatic membrane behavior, and the properties of seed coats. It must be remembered, however, that a cell which did not allow sugar to pass through its membranes for a month would find it hard to secure the needed supplies of glucose for respiration. The plasmatic membranes are permeable to all the salts and organic nutrients used in metabolism.—C. A. SHULL.

Structure of chloroplast.—For a correct understanding of the basic process, photosynthesis, it is important of course to understand the structure of the chloroplast. Much work has been done in an attempt to make clear this structure, but there are still many points about which very conflicting views are held. ZIRKLE²⁴ has made a thorough study of the chloroplast, and points out that the ordinary methods of fixing, staining, and sectioning do not give a correct understanding of the structure of the chloroplast, because the reagents dissolve out the pigments and alter the state of the stroma. Also, the pigments interfere with any attempt to study the stroma directly in living chloroplasts. ZIRKLE studied the living chloroplasts in monochromatic light. By using light of a wave length corresponding with the chief absorption band of a pigment, this pigment appeared black in transmitted light, and any pigment not absorbing this wave length was invisible. Thus, by optical means which did not alter the state of the stroma or the pigments, he was able to get rid of the pigments and study the stroma in its natural condition. He could also determine the

²⁴ZIRKLE, CONWAY, The structure of the chloroplast in higher plants. Parts I and II. Amer. Jour. Bot. 13:301-320; 321-341. 1926.

distribution of the pigments in the stroma. The inclusions of the chloroplast were studied in polarized light. For the purpose of making certain chemical tests on the chloroplasts, they were extruded into a fluid of about the same osmotic concentration, hydrogen-ion concentration, and viscosity as the cytoplasm of the cells.

ZIRKLE states that the chloroplast is a hollow flattened ellipsoid, having a central vacuole. The starch grain or grains is found in this central vacuole. It has been known for a long time, of course, that there is no sharp distinction between a leucoplast and a chloroplast; that, if exposed to light, a leucoplast may develop chlorophyll and become a chloroplast. ZIRKLE found that some of the chloroplasts of *Elodea* have as their main function the storage of starch rather than its manufacture. In function, therefore, they are leucoplasts, yet they have abundant chlorophyll and are of the same structure as the other chloroplasts. Chloroplasts of seed plants widely separated taxonomically were found to be very similar in structure.

It has long been a question of dispute as to whether there is a real membrane about the chloroplast. ZIRKLE found that there is not a real osmotic membrane. The outer layer of the stroma is somewhat denser than the inner part, and he thinks that this is due to the adsorption on the surface of the stroma of particles of cytoplasm. The apparent granules in the stroma seem to be really pores.

As to the state of the chlorophyll in the chloroplast, ZIRKLE favors the view of WILLSTÄTTER and STOLL, and others that the chlorophyll is in the colloidal state rather than in true solution in lipoids, as LIEBALDT and STERN have concluded. A lipid solution of chlorophyll is quickly destroyed by light, while ZIRKLE found that extruded chloroplasts of *Elodea* remained green for two weeks. As to whether the chlorophyll is united chemically to the stroma, as LUBIMENKO concluded, or adsorbed on the surface of the stroma, as WILLSTÄTTER and STOLL contended, ZIRKLE, as the result of enzymatic and staining studies, favors the latter viewpoint.

ZIRKLE has done much to elucidate the structure of the chloroplast, and the reviewer feels that the methods used are more likely to give correct information than the methods of many previous workers.—S. V. EATON.

Stomatal movements.—The idea that cells surrounding the guard cells are active in stomatal movements is revived by STRUGGER and WEBER,²⁵ who have studied the changes in starch content of guard cells, their companion cells, and epidermal cells of *Galium Mollugo* during open and closed condition of the guard cells. They find starch only in the guard cells in closed condition in darkness, and sometimes not even in these. After an hour of illumination, however, the guard cells possess only a trace of starch, while the companion cells contain much of it. The epidermal cells are still otherwise starch free. In several hours of illumi-

²⁵ STRUGGER, S., and WEBER, F., Zur Physiologie der Stomata-Nebenzellen. Ber. Deutsch. Bot. Gesells. 44: 272-278. 1926.

nation the guard cells are free of starch, the companion cells very full of starch, and the whole general epidermis shows starch storage. They conclude that the correlation between starch amount and stomatal condition must mean that all the cells around a stoma cooperate in the production of movement. Especially the fact that guard cells and companion cells act oppositely with regard to starch content is looked upon as significant. As starch disappears in guard cells it appears in the companion cells, and vice versa. On illumination the starch appears in the companion cells much earlier, more rapidly, and in larger amounts than in the general epidermis.

Osmotic measurements seem to substantiate a relationship. The authors point out, however, that with natural variations in light intensity the stomata undergo extremely rapid changes of stomatal aperture. They think the antagonistic sugar and starch relations of guard cells and companion cells may account for these rapid adjustments. Oddly enough, before midnight, when the stomata are completely closed, the guard cells still may have no starch, a fact noted by others. These latter observations show that the whole hypothesis is faulty.

The rapid closure of stomata has also been noted by SCARTH in a report at the Ithaca Congress. He claims that the rapid responses to light conditions are too quick to be due entirely to hydrolysis of the starch or its condensation. The diastatic action is too slow. Long before the starch undergoes any prominent change the movement is completed. He finds that illuminated and open guard cells are alkaline in reaction, probably from utilization of CO_2 in photosynthesis, and certain colloidal constituents of the cells become much swollen at the P_H developed. High turgidity in the guard cells is mainly caused by the colloidal imbibition. Closure is accompanied by increased hydrogen-ion concentration, due to accumulation of CO_2 of respiration when photosynthesis stops with low light intensity. The colloids are dehydrated as they approach their isoelectric point, the colloiddally bound water is freed, the cells quickly lose their surplus water to surrounding cells, and the guard cells close.

If hyperacidity develops due to prolonged darkness, the colloids of the guard cells may become more acid than the isoelectric point, produce an acid swelling of the colloids, and thus bring about night opening of the stomata. Here we have a mechanism whose response is almost instantaneous to light changes, and the sugar and starch reactions may be quite subsidiary to the main regulatory mechanism.—C. A. SHULL.

GENERAL INDEX

Classified entries will be found under Contributors and Reviewers. New names and names of new genera, species, and varieties are printed in **bold-face type**; synonyms in *italics*.

A

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